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THE

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THE

KANSAS UNIVERSITY SCIENCE BULLETIN.

Vol. IX, No. 1—December, 1914.

(Whole Series, Vol. XIX, No. 1.)

CONTENTS:

A Preliminary Study of the Causes that Produce "Bald-headed" Kelp, ... *Rupert Peters.*

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Vol. IX, No. 1.] DECEMBER, 1914. [Whole Series
Vol. XIX, No. 1.

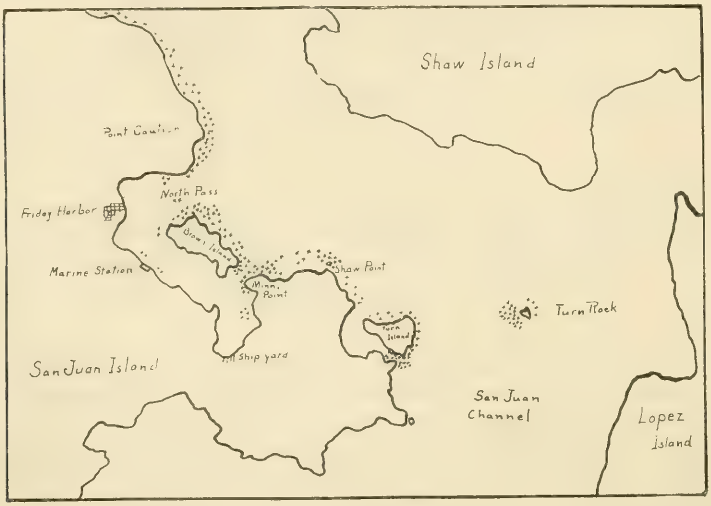
A Preliminary Study of the Causes that Produce "Bald-headed" Kelp.

BY RUPERT PETERS.

IN sailing or rowing about the San Juan Archipelago in Puget Sound, one is almost constantly within sight of the kelp beds. The typical plants have a large float, or pneumatocyst, on the surface from which many broad, flat fronds stream outward, in many cases for twelve or fifteen feet. Yet among these plants are many that have lost part or all of their fronds, and the rounded shiny pneumatocyst bulb suggests the term "bald-headed." Since the kelp is recognized as a source of potash for agricultural fertilizers, the loss of a considerable per cent of the fronds reduces the weight that might be harvested very seriously, over two-thirds in many cases.

This subject was suggested to the writer by Dr. R. B. Wylie of the University of Iowa, a member of the U. S. kelp investigation staff during the summer of 1913, following a considerable discussion of the kelp, *Nereocystis luetkeana,* and as full a statement as he was at liberty to give concerning the work being done by that staff. These obesrvations were made in connection with work at the Puget Sound Marine Biological Station, June-August, 1913. It is to be regretted that observations did not begin early in the term, since many plants were without fronds when observations began, while others were still in vigorous growth.

Facilities for getting from place to place were limited and the observations were confined almost entirely to the neighborhood of Friday Harbor, on the east coast of San Juan Island. In the accompanying sketch map the location of most of the observations made is approximately indicated, the crosses showing roughly the location and density of the kelp beds.


Shaw Island
Point Caution
North Pass
Friday Harbor
Brown Island
Marine Station
Minn. Point
Shaw Point
Turn Rock
Turn Island
Ship yard
San Juan Island
San Juan Channel
Lopez Island

Since the tide currents play an important role in the distribution and health of *Nereocystis,* a brief description of their location and strength in the neighborhood is in place. The incoming tide sweeps through the narrowed channels on either side of Turn Rock with great velocity, so much so that landings can be made there at the turn of the tide only. A branch of this current, much weakened, sweeps in south of Turn Island and around the west side. This results in the north side of the island being sheltered. The current going north to Shaw Point is not strong toward the shore, but at that point the two currents unite and sweep around it with considerable velocity. The bay between Shaw Point and Minnesota Point is sheltered. Near the latter cape the current usually divides, one branch continuing on through South Pass into Friday Harbor Bay, the other and far the stronger turning north along the east side of Brown Island. The former sweeps around through the bay and out of North Pass to join the latter at Point Caution, where they form a stiff current that hugs the shore closely for some distance to the north. With the ebb tide the directions of the currents are reversed.

With this in mind an inspection of the map shows the heaviest growth of kelp along the edges of the strongest currents, a fact already discussed by Frye, Setchell, Rigg, and others. The conditions for its growth do not belong here and have been already discussed by Professor Rigg.[1] But the currents play too important a part in this problem to be left unnoticed.

The bald-headed kelps which were under observation had lost part or all of their fronds. Usually there was a stump left of each which showed a whitened, slimy, apparently half-putrid end. (Fig. 1.) Relieved of the pull of the fronds the floats stood upright several inches out of the water, in sharp contrast to their healthy neighbors. (Fig. 2.) In many cases the side of the bulb had become flattened and wrinkled, in some cases decaying. The flattened, decaying condition was noticed in healthy plants as well, however. In the case of the latter, *i. e.*, those with a full set of fronds, this may be the result of mechanical injury, but the writer is inclined to think it due to sunburn resulting from a few hours' drying when held out of the water by neighboring plants. With the "bald-heads" it is an indication of the death of the plant, since it soon dies after losing its fronds, as proven by Zeller's experiments in 1911.[2]

The first thing to attract attention when the observations were begun was the snails, *Lacuna sp.*, present in large numbers upon many plants. The eggs were laid in jelly-like capsules, usually upon the bulb of the pneumatocyst or the bases of the fronds. The writer found them upon all parts of the plant, however, in one case a capsule upon the hapteres pulled from a depth of 39 feet. But the favorite location is as indicated above. (Fig. 3.) In feeding the snails eat the epidermis and perhaps the first cell layers beneath. As the meristem region is youngest and presumably tenderest, or perhaps because it is exposed to the air, it is the region most frequently chosen for a meal. Doctor Setchell[3] says, "The central or medullary tissues are made up, as a rule, of elongated—often tubular—cells and have for their function the conduction of substances manufactured and distributed from the outer tissues. They are destitute of assimilating cell organs, the chromatophores or chlorophyll granules. The outermost layers are composed of smaller cells, nearly isodiametric, which contain the chromatophores and are consequently the assimilating tissues." This led the writer to question the effect of these masses of eggs on the meristem region; could they cut off light enough to interfere with growth or enough to weaken the cells to the point that the fronds would drop off at that point?[4] A large number of observations were made off the east side of Brown Island, several plants which had most of the meristem region covered by egg clusters being under observation for over three weeks. No change could be discerned and the fronds seemed as tough at the close of the period as at the beginning. Professor Rigg's[5] statement that "the region of elongation in this plant extends over the pneumatocyst and the bases of the fronds" has since come to notice and explains the failure to get results. The egg masses were over the bases of the fronds and the top only of the pneumatocysts, leaving most of the "region of elongation" exposed.

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[1] Professor Rigg, Bul. P. S. Marine Sta. Notes on the Ecology and Economic Importance of *Nereocystis luetkeana*.

[2] Professor Rigg, Fertilizer Resources of the U. S., Appendix L.

[3] Doctor Setchell, same, Appendix K.

At the same time as the above, observations were made to determine the effect of the scorings made when the snails fed. (Fig. 4.) Three healthy plants were selected and tagged. The cuticle was scraped with a knife blade from one side of the base of one cluster of the fronds of the first, from one side of both clusters of the second, and from both sides of both clusters of the third. A fourth was freshly snail-eaten at the same point, the animal having been brushed off in order to see the extent of the damage. These four plants and others were under observation for a month. The cuts did not seem to weaken the fronds in the least. No breaks came and they were healthy until the snails ate them up, that is, peeled so much off in large spots that the fronds were weakened and broke off in fragments. (Fig. 5.) The failure to get positive results from the experiments and from the observations of the snail's feeding habits with this one point in mind nevertheless gave positive evidence as to the loss of fronds in some plants. On the thick beds where the snails were abundant, they ate the fronds in holes, weakening them so that they broke off in large pieces. The snails seemed to retreat before the impending loss and to continue eating toward the base until the whole frond was gone. The appended table of some observations gives color to this conclusion.

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[4] Since the above was written, Doctor Wiley has suggested that the interference with gaseous exchange by the excretions of the snails might be of more importance than this interference with light relations.

[5] Professor Rigg, Fertilizer Resources of the U. S., Appendix L.

From the table it is seen that the snails are found on the kelp where the current is weak, that in these places many plants are without fronds, that the kelp in the strong currents have relatively few without fronds and that where they do occur they are in clustered groups, and that where the plants are scattered and in strong currents they are uniformly healthy.

In observation No. 23 the plants were against the shore, so that as the tide ebbed and flowed they were swept back and forth across the rocks. This may account for some being without fronds. The same condition was noticed along the east side of the island and also off Point Caution. The plants next the shore were nearly all frondless. In each of these cases the shore is abrupt. South of Turn Island the shore is gently sloping and the plants there, similarly placed to those in the places just mentioned, were healthy.

Yet these observations have not accounted for the numbers of bald-heads in the strong currents. Drifting back to the Station after making observation No. 26, the writer's attention was caught by some fronds in the thick clusters west of Shaw Point which had been thrown up over the floating pneumatocysts of neighboring plants and, exposed to sun and wind, had become discolored and dry. However, they were not crisp enough to break. Yet this suggested a new factor and it was late when the Station was reached. Several like cases were found that evening. The field from below Turn Island to Point Caution was resurveyed with this point in mind and thirty-six similar cases found, all being of plants in the center of thick clumps. Some were so crisp as to break off like dead leaves when handled. Unfortunately, bad weather prevented watching these closely. So in the quiet water near camp a fine healthy plant was selected, exposed to tide currents and the wash from every passing vessel. A rude raft of boards was built about it and the fronds were thrown up over this in the same manner as had been observed in the field. A few were left floating as a check. On account of the wash from vessels it was very difficult to keep them as desired without tying the fronds themselves, which would have introduced a new factor. Yet on the third day the exposed fronds were discolored and soon became crisp, although somewhat leathery. Some were placed back in the water to determine if they would regain their former elasticity. Absence from camp prevented observations for three days, but at the close of this time the fronds had broken off to within eight inches of the pneumatocyst and their ends showed the characteristic white, decayed appearance to be found on all bald-heads. (Fig. 6.) The check fronds seemed as healthy as ever and the dried ones that had been returned to the water had broken off, save one, and it broke when an attempt was made to lift it. It is to be regretted that time did not permit a further test and that the weather prevented field observations; however, it is very suggestive as to the causes of the bald-heads in Nos. 4, 15, 11, 19, etc.

In the quieter waters, the upper portion of the stipe and the pneumatocyst of many plants were found covered with a dense growth of *Ulva fasicata,* this appearing on a greater number of plants each week as the season progressed. So far as could be determined it caused no injury to the plants, even though they were sometimes fairly hidden by it. *Enteromorpha* was frequently found in the same situation. *Antithamnion americana* and *Porphyra nereocystis* were common on the deeper part of the stipe. Various *Rhodophyceæ* shared the holdfasts with many specimens that were pulled up. Other browns were often attached to the same stones as were the *Nereocysti.* In the quieter waters the stipes and pneumatocysts were slimy with a heavy growth of diatoms. Many fronds were spotted with the colonies of a Bryonzoan, *Membraniphora membranceæ,* in many cases almost hidden by its white spots. (Fig. 7.)

None of these, however, seem to cause the damage. So far as observations have gone, the loss of the fronds is due to (1) being eaten off and weakened by the snails; (2) to the drying action of sun and wind when cast up over other plants by the waves and currents and so exposed; and possibly (3) to friction against the rocky shores. It is recognized that these are but tentative answers to the problem. A longer period of observation and through a whole season, as well as over a larger territory, is necessary to verify or disprove these conclusions.

NOTE.—The writer has spent three seasons at the Puget Sound Marine Biological Station as a member of the Kansas party led by Professor W. J. Baumgartner of the State University. To his unselfish interest the opportunity for the above work was largely due.—R. P.

NUMBER AND LOCATION.	Current.	No. of Plants.	Grouping.	Number bald.	Snails.	Remarks.
1—East of Brown Island	Strong	28	Scattered	None	None	A few Bryozoa.
2—East of Brown Island	Strong	21	Scattered	None	None	
3—East of Brown Island	Strong	12	Scattered in deep water	None	None	Fine specimens.
4—East of Brown Island	Strong	53	Close cluster	7 in center of group.	None.	
5—East of Brown Island	Weak	64	Scattered	22, with 30 others badly eaten	Many	
6—East of Brown Island in sheltered cove	Weak	188	Scattered	168	Many	Not an injured plant in the cove.
7—Cove False Bay	Weak	46	Crowded	18	Many	
8—Outside No. 7	Strong	97	Crowded	None	None	
9—See note below.						
10—East of Minn. Point	Strong	4	Scattered in deep water	None	None.	
11—East of Minn. Point	Strong	43	Crowded	9 in center of group	None	Fine plants; none sick; but the bald in the center.
12—East of Minn. Point	Strong	26	Scattered	None	None.	
13—East of Minn. Point	Strong	3	In deep water	None	None.	
14—East of Minn. Point	Strong	5	In deep water	None	None.	
15—North of Minn. Point	Strong	67	Close clump	11 in center	None	Similar to No. 11.
16—West of Minn. Point	Weak	122	Scattered	69 on shore side, with 42 others badly eaten	Many	Little current on shore side.
17—West of Shaw Point in bay	Little	131	Scattered	81	Few	Shallow water.
18—North of Shaw Point	Strong	178	Dense cluster	16	None	
19—East of Shaw Point	Strong	87	Crowded	11	None	Many plants kept under by the strong current.
20—See note below.						
21—South toward Turn Island from Shaw Point	Moderate	53	Scattered	3	Few.	
22—North of Turn Island	Weak	22	Scattered	None	None	Out from shore.
23—North of Turn Island	Weak	41	Scattered	28	None	Against shore.
24—South of Turn Island	Moderate	28	Crowded	4	None	
25—South of Turn Island	Moderate	73	Crowded	8	Few	
26—West of Turn Rock	Strong	164	Crowded	2	None	The prettiest kelp bed seen.
27—North of Brown Island middle of North Pass	Strong	38	Scattered	None	None	Kept under water most of the time.
28—South of Point Caution	Weak	81	Crowded	65	Many	Snails found even on the hapteres.

29—South of Cattle Point toward the Salmon Banks were a great number of single plants in tide currents so strong as to keep them almost constantly under. These were, without exception, healthy plants.

30—A similar condition to that in No. 29 existed across the channel north of Anacortes.

31—At the west opening of the passage between Orcas and Shaw Islands a clump is swept to and fro by the changing tide currents; the plants are big and healthy; no snails.

9—From False Bay Cove to Kanaka Bay is a dense growth with a strong current outside and a weak one next the shore. Near shore were many plants with fronds missing and with many snails. Outside, very few blades were missing and snails were not to be found.

20—The condition east of Point Caution is practically the same as in No. 9, save that the growth of kelp is not nearly so extensive.

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CONTENTS:

A LIST OF THE DESCRIBED SPECIES OF FOSSIL AMPHIBIA, . *Roy L. Moodie.*

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VOL. IX, NO. 2.] DECEMBER, 1914. [WHOLE SERIES VOL. XIX, NO. 2.

A List of the Described Species of Fossil Amphibia.

ROY L. MOODIE.

Department of Anatomy, University of Illinois, Chicago.

SOME of the species listed below may be regarded as reptiles, but most of the species herewith given certainly belong to the amphibian phase of vertebrate development. My excuse for publishing such a list is that no such list exists and it will undoubtedly be of service to some one in the future who undertakes the examination of these forms. The list was prepared in connection with my studies of the Carboniferous Amphibia of North America.

No attempt has been made to construct a complete catalogue of the species of fossil Amphibia, showing synonomy and indicating all references. This will be left to others. The list will serve the purpose of showing the geographical distribution of fossil Amphibia and the number of species described, as well as indicating the original place of description of each species. There is no doubt that many of the species listed below are synonyms, but in the present imperfect state of our knowledge of the species a correct arrangement of them would be almost impossible and nearly worthless. The list is written primarily with a view to a revision of the genera and species of the fossil Amphibia of the world and is published here as

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Received for publication, March 22, 1914.

an aid to those who may have occasion to use it. The list given below is believed to be fairly complete up to June, 1912.

Acanthostoma vorax Credner. Permian of Saxony. Credner, 1883—Zeit. d. Deutsch. Geol. Gesell., p. 288.

Acheloma cumminsi Cope. Permian of Texas. Cope, 1882—Proc. Amer. Phil. Soc., p. 456.

Actinodon brevis Gaudry. Permian of France. Gaudry — Nouvelles Archiv. Mus. Hist. Nat. (2), X, pp. 1-32.

Actinodon latirostris Jordan. Permian of France. Von Meyer—Paleontographica, Bd. VI, p. 211.

Adenoderma gracile Fritsch. Permian of Bohemia. Fauna der Gaskohle, Bd. I, p. 126, Taf. 19, fig. 1.

Algeinosaurus aphthitos Case. Permian of Texas. Case, 1911—Carnegie Institute Publication, No. 146, p. 60.

Amblyodon problematicus Dawson. Carboniferous of Nova Scotia. Dawson, 1882—Phil. Trans. Royal Soc., London, p. 644.

Amphibamus grandiceps Cope. Carboniferous of Illinois. Cope, 1865—Proc. Acad. Natl. Sci., Phila., p. 134.

Amphibamus thoracatus Moodie. Carboniferous of Illinois. Moodie, 1911—Proc. U. S. Natl. Museum, 40, p. 431, fig. 2.

Anaschisma browni Branson. Triassic of Wyoming. Branson, 1905—Journ. Geol., xiii, p. 585, figs. 1-8.

Andrias scheuchzeri, Tschudi. Miocene of Switzerland. Von Meyer, 1845—Fauna der Vorwelt, Bd. I, p. 28, pl. 10.

Andrias tschudii von Meyer. Miocene Lignite of Switzerland. Von Meyer—Paleontographica, Bd. 7, p. 49, pl. ix.

Anisodexis enchodus Cope. Carboniferous of Ohio. Cope, 1885—Proc. Amer. Assn. Adv. Sci., p. 406.

(This species probably belongs in the genus *Sauropleura*, there is no assurance that it belongs in *Anisodexis*.)

Anisodexis imbrecarius Cope. Permian of Texas. Cope, 1882—Proc. Amer. Philos. Soc., xx, p. 459.

Anthracerpton crassosteum Owen. Carboniferous of England. Owen, 1865—Geol. Mag., Dec. I, Vol. II, p. 6.

Anthracosaurus edgei Bailey. Carboniferous of England. Bailey, 1875—Rept. Brit. Assn., p. 62.

Anthracosaurus raniceps Goldenburg. Carboniferous of Germany. Goldenburg, 1873—Fauna saraepontana fossilis, heft 1, p. 4, taf. 1, fig. 1.

Anthracosaurus russelli Huxley. Carboniferous of Scotland. Huxley, 1863—Q. J. G. S., xix, p. 56, fig. 1.

Apateon pedestris von Meyer. Carboniferous of Germany. Von Meyer, 1844—Neues Jahrb. f. Mineral., p. 336.

Aphaneramma rostratum Woodward. Triassic of Spitzbergen. Woodward, 1904—Proc. Zool. Soc., London, II, p. 173.

Archegosaurus austricus Makowsky. Makowsky, 1876—Sitz. k. Akad. wiss. Wien. I, lxxiii, hft. 3, p. 155-166.

Archegosaurus decheni Goldfuss. Upper Carboniferous of Germany. Goldfuss—Paleontographica, Bd. vi, p. 69-211, pl. xi-xxiii.

Archegosaurus latifrons Geinitz und Deichmüller. Permian of Saxony. Paleontographica, Bd. xxix, p. 21, pl. vi.

Archegosaurus ornatus Woodward. Permo-carboniferous of India. Paleontologia Indica, 1905—N. S. II, p. 11, pl. x.

Aspidosaurus apicalis Cope. Permian of New Mexico. Cope, 1881—Amer. Natl., xv, p. 1020.

Aspidosaurus chiton Broili. Permian of Texas. Broili, 1904—Paleontographica, Bd. LI, p. 40.

Aspidosaurus crucifer Case. Permian of Texas. Case, 1903—Journ. Geol., xi, p. 399.

Aspidosaurus glascocki Case. Permian of Texas. Case, 1901—Bull. Amer. Mus. Natl. Hist., xxviii, p. 179.

Aspidosaurus novomexicanus Williston. Permian of New Mexico. American Permian Vertebrates, 1911, p. 12, pl. xxxviii, fig. 1.

Aspidosaurus peltatus Williston. Permian of New Mexico. American Permian Vertebrates, 1911, p. 13, pl. xxxii, fig. 7.

Baphetes minor Dawson. Carboniferous of Nova Scotia. Dawson, 1870—Q. J. G. S., xxvi, p. 166.

Baphetes planiceps Owen. Carboniferous of Nova Scotia. Owen, 1854—Q. J. G. S., x, p. 207, pl. ix.

Batrachiderpeton lineatus Hancock and Atthey. Carboniferous of England. Hancock and Atthey, 1870—Ann. and Mag. Natl. Hist., 4th series, VI, p. 56, pl. 1.

Batrachosaurus (*Saurosternon*) *hainii* Huxley. Trias of South Africa. Huxley, 1868—Geol. Mag., ser. 1, v. p. 201. Owen, 1876—Cat. Fossil Rept. South Africa, p. 69, pl. 70.

(This species was regarded by Huxley as a reptile allied to *Telerpeton*, but Owen later referred it to the Labyrinthodonts.)

Batrachosuchus browni Broom. Karoo beds of South Africa. Broom, 1903—Geol. Mag. (iv), x, p. 1.

Bothriceps australis Huxley. Triassic of Australia. Huxley, 1859—Q. J. G. S., xv, p. 647, pl. xxii.

Bothriceps huxleyi Lydekker. Karoo beds of South Africa. Lydekker, 1889—Ann. Mag. Natl. Hist., ser. 6, IV, p. 476.

Bothriceps (*Petrophryne*) *major* Owen. Karoo Beds of South Africa. Owen, 1876—Cat. Fossil Reptiles South Africa, p. 68.

Bothriceps woodwardi Moodie. Carboniferous of Australia. Moodie, 1910—Amer. Natl., xliv, p. 367.

Brachydectes newberryi Cope. Carboniferous of Ohio. Cope, 1868—Proc. Acad. Natl. Sci., Phila., p. 214.

Brachyops laticeps Owen. Mangli Group (Jurassic?) (Trias) of India. Owen, 1854—Q. J. G. S., X, p. 473, XI, p. 37, pl. ii.

Branchiosaurus ambystomus Credner. Permian of Saxony. Credner, 1881—Zeit. d. Deutsch. Geol. Gesell., p. 574.

Branchiosaurus caducus von Ammon. Permain of Germany. Von Ammon, 1889—Permische Amphibien, p. 81, pl. iv, fig. 1.

Branchiosaurus (*Protriton*) *fayoli* Thevenin. Carboniferous of France. Thevenin, 1906—Ann. de Paleont., I, pt. 3, pp. 1-19, pl. 1.

Branchiosaurus moravicus Fritsch. Permain of Bohemia. Fritsch, Fauna der Gaskohle, Bd. I, p. 82, Taf. 7, figs. 1-5.

Branchiosaurus (*Protriton*) *petrolei* Gaudry. Permian of France. Gaudry, 1875—Bul. Soc. Geol. de France, p. 300, pl. 7-8.

Branchiosaurus robustus Fritsch. Permian of Bohemia. Fauna der Gaskohle, Bd. I, p. 84, Taf. 10, figs. 8-10.

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On the Anatomy of *Grindelia Squarrosa.*

BY NORA E. DALBEY.

Plates II-VII.

GRINDELIA SQUARROSA, popularly known as gum plant, is a widely distributed representative of the order Compositæ (4, 12, 13). In many respects it is a typical xerophyte. It is very hardy, with structural characteristics which enable it to withstand drouth, and with a habit of forming rosettes in the spring, which presumably helps it to withstand early frosts. With the exception of fungi, it has few natural enemies (8, 9).

A striking external characteristic is the presence of an aromatic gummy exudate over the entire surface. This exudate, which is secreted by glands, is excreted in great quantities from the flowers, while the glands on the leaf and stem always excrete an amount sufficient to impart a glutinous character very perceptible to the touch.

The plant presents some unusual structural features; and although it has been studied ecologically, but little anatomical work has been done upon it. Beauvais (1), in 1889, published an article on *Grindelia robusta.* His paper treats of the structrue as well as the chemistry of the plant. He calls the substance excreted from the epidermal glands a resin, similar to that found in the resin ducts in the interior collenchymatous layer.

Glasford (7), in 1898, published his work on the chemistry and histology of *Grindelia robusta.* He mentions resin glands

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on the stem, leaf, and involucre scales, also fragments of resin found in the flower. He characterizes the substance as very slightly soluble in water, readily soluble in benzene, alcohol, ether and chloroform, showing an acid reaction when in solution, and combined with the alkalies yielding an amorphous compound, which has the properties of resin soap.

THE LEAF.

The structure of the leaf was determined from specimens preserved in chloral hydrate, bleached in potassium hydroxide, and mounted in glycerine, and from material preserved in formalin, imbedded in paraffin, sectioned and stained with a double stain of safranin and Delafield's hæmatoxylin.

Anatomically the leaf presents some striking features. It averages 2.3 cm. in length, is rather thick and of a dull gray color, the dullness probably caused by crests and ridges of the cuticle.

When bleached it has the appearance of a pellucid network, due to the unusual character of the leaf tissues. (Figs. 1 and 3.) Water-storage tissue, made up of relatively large parenchyma cells, surrounds masses of other mesophyll cells that contain chloroplasts, but are not differentiated into palisade and spongy tissues. (Figs. 3, *m* and *h;* 5, *u* and *v;* and 6, *c* and *d.*) These chlorophyll-bearing cells resemble palisade tissue more than spongy tissue in both form and arrangement. Warming (18) mentions the fact that palisade tissue is greatly developed in xerophytes, either by an increase in the number of layers or in the height of the cells, and also mentions that there is a difference of opinion as to the significance and cause of this structural feature. He regards regulation of transpiration as the most essential reason for the structural differences, with light as a very important factor.

Clements (5) suggests that when the photosynthetic cells are all palisade cells, the condition may have been brought about by low water content.

The water-storage cells surround each vascular bundle, and extend to the epidermis above and below, thus giving the leaf the appearance of a network of large cells surrounding areas of smaller-celled tissue (fig. 5, *v* and *u*). A remarkable characteristic of the leaf is its capacity to hold water, for the water-storage cells occupy about one-third of the volume of the leaf (fig. 2, *e*).

There are a few thick, bud-shaped hairs on the margin of the leaf, which extend toward the apex, and project almost parallel with the margin of the leaf (figs. 13, *b*, and 14, *g*).

The chloroplast-bearing mesophyll cells are irregular in arrangement, some patches extending from surface to surface and others not (figs. 3 and 5). They vary from .006 mm. to .011 mm. in width, and from .016 mm. to .030 mm. in length. A single cell shows 8 to 16 chloroplasts of the usual type (fig. 17, *s*).

The leaf has a clasping base, and is entered by three vascular bundles. Near the base of the leaf, the tissue between these three bundles is composed of water-storage cells (figs. 1, *a*, and 7, *i*).

In cross section the vascular bundles are seen near the center of the leaf, midway between the upper and lower surfaces (figs. 3, *i*, and 5, *w*). The number in one cross section varies from 10 to 36.

The marginal veins unite in the serrations and form a coalescent mass of tracheids (figs. 1, 13, *c*, and 14, *i*). The tracheids have bordered pits, and spiral thickenings (fig. 20, *b* and *c*). The xylem and phloem are about equal in amount in the larger veins. They are separated by a layer of conjunctive parenchyma varying from one to three cells in thickness (fig. 4, *q*). The number of tracheids found in a cross section of a single vascular bundle varies from 5 to 37, and in the larger veins wood parenchyma and tracheal tissue alternate in radial rows, and bast fibers subtend the base of each bundle (fig. 4).

Resin ducts, on the average .028 mm. in diameter, are found near the phloem. They are surrounded by from 7 to 12 secreting cells (figs. 3, *l*, and 5, *y*).

The cells of the epidermis are rather thick-walled, and appear largest when seen from the surface (fig. 15, *k*). The outer walls are decidedly thickened, being on the average .007 mm. thick. A cuticle layer .0045 mm. thick is sculptured into irregular ridges, and in cross sections in places appears to be striated. In addition to this, cutinization goes deeper to the extent of .002 mm. in the walls of the epidermal cells which join the water-storage tissue.

A cuticle layer covers the walls of the guard cell where they bound the stomata, and extends back over the inner epidermal walls, thus lining the outer part of the stomatal chamber (fig. 19, *a*). The epidermal cells are rich in protein, and in the young leaf they contain a small amount of starch.

The stomata are of the usual type (fig. 18). They are more numerous on the upper surface and over the areas of the chloroplast-bearing mesophyll (fig. 15), there being on the average 126 to the sq. mm. on the upper surface, and 97 to the sq. mm. on the lower surface. The guard cells are comparatively small, and are slightly raised above the surface of the epidermis (fig. 19). Such a position may be of physiological importance, since it increases the depth of the stomatal opening. Brown and Escombe (2) have shown by their experiments that whenever there is a constant difference maintained between the partial pressure of the vapor inside and outside the leaf, the amount of diffusion of water vapor is controlled by the depth of the apertures, and not by their areas, the rate of diffusion varying inversely with the depth of the stomatal opening. Thus the increased depth of the stomatal tube would decrease diffusion, which decrease may, however, be partly offset by the vapor density shells being in such a position that they are readily removed by wind.

Resin glands occur on the surface of the leaf, on the average 12 to the sq. mm. on the upper surface, and 3.4 to the sq. mm. on the lower surface. (Fig. 12.) They increase in number from the base of the leaf to its apex, and occur in clusters, often as many as thirty in a group at the apex, and near the tip of each tooth on the margin. (Fig. 14.) On the surface of the leaf the glands are usually sunken to a depth of about one-fourth the thickness of the leaf, and are always found in connection with the water-storage cells which surround the veins and extend to the epidermis. The layer of cells beneath the glands are usually elongated, and have comparatively thin walls (fig. 11, *u*) without a cuticle layer.

In cross section the glands are round, with an average diameter of .045, and are made up of from 20 to 60 cells. (Fig. 12, *a*.) A longitudinal section of a gland, however, presents three to four tiers of cells, with the outer layer very regular in form and arrangement. (Fig. 13, *b*.) The cells are rich in cell contents, and have prominent nuclei. On the surface of the leaf each gland has a thick coating of gum-resin, in the form of a minute droplet, while on the margin the entire cluster is covered by the same gummy excretion. On the upper surface, 9.4

per cent of the surface area is covered by these droplets, and on the lower surface 2.6 per cent. This gum-resin has a composite character. It swells and is slightly soluble in water, the solution showing an acid reaction. When a dry leaf was put into hot water and left for twenty-four hours the gum disappeared from the surface of the leaf, and that on the margin became viscous. Some of this gum-resin from the margin, after soaking in water twenty-four hours, was put in ether ten minutes, but did not dissolve. It is partially soluble in ether, alcohol and xylene, and when a small amount, placed on a slide, was irrigated with ether for a period of about ten minutes, a part dissolved, and the residue gave the reaction for gum when treated with methylene blue.

The substance shows the characteristic red stain when left twenty-four hours in a solution of alcanin, or in Sudan III, but it failed to show a resin reaction with copper acetate.

It may be possible that the tracheids at the margin of the leaf (fig. 13) function as water-storage cells, the resin serving to prevent transpiration in time of drouth; however, this is not a very satisfactory explanation of the relationship of the glands on the surface to the conducting and water-storage systems of the leaf. (Figs. 3, *k*, and 14.) It seems more probable that these glands function as organs of absorption. Kerner and Oliver (10) cite the case of an Aizoon having epidermal glands which serve to collect water and transfer it to the cells within the leaf. The gum resin, which swells in water, may be able to absorb the water from the rain and dew and transfer it, by osmosis, into the cells of the glands, whence it might pass into the noncutinized elongated cells of the epidermis and on into the adjoining water-storage tracheids (fig. 13), or into the water-storage cells of the mesophyll (figs. 3, *k*, and 11, *u*), and from these storage cells it could be slowly given off to the plant in time of drouth.

THE STEM.

The stem has a gray-green color, with a minutely roughish surface. In cross section it is round, with the outline broken by from three to eight protuberances, which occur at regular intervals. (Fig. 25.) Glands similar to those found on the leaf are distributed irregularly over the surface of the stem. The cross section shows a circle of open collateral bundles varying from ten to thirty-four in different sections. The stem is mechanically strong, due to a large amount of well-developed supporting tissue, and an absence of large intercellular spaces. It contains a rather large proportion of conductive tissue. It is interesting to note here that in the publication by W. A. Cannon (2) on "Water Conducting Tissue of Some Desert Plants," he cites an experiment in which irrigated plants were poorer in conductive tissue than stems of the same diameter from non-irrigated plants.

Each stem bears a head of flowers, and a cross section at the base of the head shows a collenchyma zone fifteen to twenty cells deep. The collenchyma contains relatively large resin ducts .045 mm. in diameter. The resin glands are more numerous at the base of the flower than lower down on the stem.

The pith, comprising 20.3 per cent of the stem cross section, is composed of parenchymatous cells with comparatively thin and delicately pitted cellulose walls. (Fig. 23.) In cross section the pith cells vary in diameter from .015 to .067 mm. The walls of a layer of pith cells adjacent to the wood zone often become lignified. In the mature stem the pith cells are not empty, but contain some protein, a little starch, and numerous clusters of calcium oxalate crystals, made up of long, slender crystals, which give the cluster a spiny appearance. These crystals sometimes occur singly.

In the pith, and dispersed through the cortex and the wood parenchyma, there occur round to oval vesicles in which are minute globules that are stained a bright red when left for 24 hours in alcannin or in Sudan III. When sections were put into xylene and left 24 hours they did not show this stain reaction with alcannin and Sudan III. After being treated with xylene, the vesicles are present, and appear to be plastids which manufacture and store up oil.

The tracheal elements are in radial rows separated by relatively broad zones of wood fibers and parenchyma. (Fig. 22.) The large ducts occur in greater number near the inner part of the wood zone (fig. 26, *w*). In transverse section the vessels are round to oval in shape. The tracheal tubes and the tracheids have bordered pits, sometimes slightly elongated, but not enough to justify the use of the term scalariform (fig. 29, *g*), and single, double and triple spiral thickenings occur on the earlier vessels (fig. 29, *h*).

The ducts, the tracheids, the wood fibers, and the ray cells of the xylem have lignified walls. One bundle of average size contained 176 vessels, of an average diameter of .11 mm. The vessels compose 3.74 per cent of the stem. The wood parenchyma is scanty in amount. It occurs in radial rows between the vessels and surrounds the inner margin of the bundles. (Fig. 26, *y*.) These cells contain granules which stain yellow with chlor-zinc-iodide. The wood fibers are of the characteristic type. (Fig. 26, *v*.) They average .0067 mm. in diameter, and are from .045 mm. to .150 mm. long. The walls, on the average .0013 mm. thick, are lignified and distinctly marked by slit-shaped straight pits.

The line of demarcation between the xylem and the phloem takes a sinuous course, as seen in fig. 25. In the stem near the base of the flower there is an equal amount of xylem and phloem, but in the portion lower down on the stem the ratio of xylem to phloem is 10 to 1. The phloem group is made up of small irregular cells with thin walls (fig. 30), and there is no differentiation into sieve tubes and companion cells. They gradually merge into the cambium layer. (Fig. 22, *i*.) In irregular masses the stain reaction with aniline sulphate and with safranin is characteristic of lignified walls. A longitudinal section shows lignification, extending in some cases the length of several cells and in other cases only part of the length of a single cell.

A complete cambium ring is not discernible.

The medullary rays in stem cross sections are narrow linear zones from two to six cells wide. Their distinguishing feature is a slight radial elongation of the cells. (Fig. 27, *b*.) The ray cells of the xylem are lignified, and at the sides they are bordered by a mass of xylem parenchyma cells resembling wood fibers in arrangement and in thickness of cell walls. (Fig. 27, *c*.) In longitudinal section these are brick-shaped, with simply pitted walls, and are usually empty of cell contents.

Within the pericycle exterior to each bundle is a mass of bast tissue. (Figs. 22, *n*, and 25, *s*.) Each bast group is radially from five to eight cells in thickness, and its cells are usually pentagonal in cross section. (Fig. 31.) The cell walls, on the average .004 mm. thick, are lignified, and are marked by straight pits. The average length of the bast fibers is about .11 mm., with long, tapering, interlacing ends.

The endodermis is very conspicuous. (Fig. 36, *p*.) In cross section the cells are round to oval and from .007 mm. to .045 mm. thick. In longitudinal section they are brick-shaped, on the average .088 mm. in length, and show a distinct nucleus. (Fig. 37, *s*.) These cells contain protein, and a small amount of starch. Sections stained twenty-four hours in alcannin and in Sudan III and washed with fifty per cent alcohol show all the walls heavily suberized with the exception of from one to three cells bordering the thin-walled parenchyma cells between the bast groups. (Fig. 36.) These cells have comparatively thin cellulose walls, and they are clearly places of radial conduction.

The central band of the cortex is composed of collenchyma cells with an average diameter of .022 mm. and an average length of .120 mm. (Figs. 25, *q*, and 34, *j*.) They have little uniformity in size and shape, with the exception of one layer which lies adjacent to the epidermis and resembles the epidermis in structure and in cell contents. (Fig. 34.) The cellulose walls contain straight pits. Stomata occur on the stem in connection with rather large aërating spaces. Resin ducts occur adjacent to the bast. They are in cross section oval to round in shape, .015 mm. to .045 mm. in diameter, and are surrounded by from seven to twelve secreting cells rich in cell contents.

The epidermis consists of one layer of cells, which have thick cellulose walls. The outer wall is .007 mm. thick and the other walls each .0015 mm. thick. The lumen of the cells is nearly round, and has an average diameter of .011 mm. The epidermal cells contain chloroplasts of the usual type, and granules which show the characteristic stain reaction for protein. There is a very marked cuticle .0045 mm. thick, with deep ridging, which often accompanies great thickness. Stomata the same in structure as the stomata of the leaf are scattered over the surface, on the average nine to the sq. mm.

The inflorescence is a head surrounded by an involucre of spirally arranged bracts. Before the bud opens a large drop of gum-resin is excreted, which covers the buds with a waxy coating. Small disk flowers occur in the center of the head, surrounded by a circle of golden-yellow ray flowers. A transverse section through the top of one of the involucre bracts is almost round, with a diameter of .52 mm.

Toward the base of the bract the outline approaches triangular, being flattened on one side. Vascular bundles consisting of a large amount of phloem (fig. 35, *l*) occur near the central part of the bract, and in cross section are arranged in the shape of an arch. (Fig. 35.) The upper part of the bract contains large masses of mesophyll cells separated by water-storage tissue, while the lower part is made up of more scattered masses of mesophyll and a larger amount of water-storage parenchyma tissue, which often contains large aërating spaces. Resin ducts are found in the parenchyma adjacent to each bundle, the central one reaching a diameter of .03 mm. The mesophyll cells appear to contain more chloroplasts than the mesophyll of the leaf.

The epidermis of the bract is the same in structure as the epidermis of the leaf, and contains stomata and a large number of resin glands similar to those on the leaf. There is a thick cuticle layer covering the epidermis.

SUMMARY.

1. The stem, leaves and involucre scales are covered with an aromatic sticky exudate.

2. This exudate comes from multicellular superficial glands that occur as frequently as 1.2 to the sq. mm. on the upper surface and 3.4 to the sq. mm. on the lower surface of the leaf.

3. The exudate appears to be essentially a gum-resin, some of its substance being soluble in water, some in ether, alcohol, and in xylene, and it stains as mucilage does with methylene blue, and as oils and resins do with alcannin and Sudan III.

4. Where the glands occur at the tips of the marginal serrations clusters of tracheids extend to them from the marginal veins, and where they occur over the surface of the leaf they are subtended by masses of water-storage cells.

5. These water-storage cells compose about one-third of the mesophyll; they surround the veins, and often extend from one surface to the other.

6. It would seem from their position and the nature of their exudate that the glands might serve in the absorption of water from dew or rain. The gum of the exudate could imbibe the water and give it over to the storage tracheids and parenchyma, and the resin could protect against evaporation.

7. Resin ducts occur adjacent to the veins of stem, leaves and flowers.

8. The leaf is entered by three vascular bundles, and near the base the tissue between the veins is composed of water-storage parenchyma.

9. The chloroplast-bearing cells of the mesophyll are not differentiated into palisade and spongy parenchyma.

10. In the phloem of the stem there is no differentiation into sieve tubes and companion cells, and irregular patches of thin-walled phloem cells show more or less lignification of the walls.

11. In the stem the cell walls of the conspicuous endodermis are suberized, excepting from one to three cells that border the thin-walled parenchyma of the pericycle standing between the bundles of bast fibers. These uncutinized passage cells of the endodermis are undoubtedly places where translocation of materials takes place between the outer and inner bark.

12. The stem is made strong by a large development of wood, bast and collenchyma.

The material selected for this investigation was obtained from Scott county, Kansas, in midsummer, at the height of the flowering season. The study of the anatomy was carried on in the research laboratory of the botanical department of the University of Kansas, under the direction of Prof. W. C. Stevens, to whom his student gratefully acknowledges her appreciation of his help and interest. Thanks are due to Mr. L. M. Peace, preparator and demonstrator in the botanical laboratory, for his invaluable suggestions and assistance during the progress of the investigation.

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CONTENTS:

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A Study of the Histological Variations of *Quercus muhlenbergii.*

BY JOHN A. ELLIOTT.

Plates XI to XV.

THE work, the result of which this paper will set forth, was done upon the suggestion and under the helpful direction of Prof. W. C. Stevens. Four native oaks growing in close proximity and similar in many characteristics showed some differences which made their classification rather doubtful. A study of the anatomy was undertaken as a more definite means of detecting constant similarities and variations.

One of the four filled the description of *Q. muhlenbergii* in Gray and Britton and Brown. The other three did not fit satisfactorily any description, so that in the study all three were designated by numbers, as 1, 2, 3 and 4. Specimens of each sent to the Gray Herbarium, Harvard University, were returned as all being *Q. muhlenbergii.* This made the designation by number as good as any as a basis for description.

MACROSCOPIC DIFFERENCES.

The leaves of No. 1 averaged smaller than those of the other trees and were more uniform in shape and size. The teeth of the serrate margin were sharply acuminate, averaging about ten on a side. The secondary veins were almost straight. The leaves of Nos. 2, 3 and 4 varied greatly in size and form, the teeth never being as sharply acuminate as in No. 1, and the leaves, especially of Nos. 2 and 3, being gen-

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erally much larger. The secondary veins of No. 4 were nearly straight, those of No. 2 and 3 were curved. (Figs. 1 to 7.)

The acorns showed the most marked macroscopic difference between the four oaks. The acorns of No. 1 averaged 1.5 cm. in length and width; No. 2 averaged 2 cm. long by 1.5 cm. in width; No. 3 averaged 1.1 cm. in length by 1.2 cm. in width; No. 4 averaged 1.6 cm. in length by 1.2 cm. in width. (Figs. 8, 9, 10, and 11.) Excepting No. 4, the cups were much alike, being relatively deep and covered with thick bracts. No. 4 was shallow and covered with thin, tightly appressed bracts.

The seeds varied in size in about the same ratio as the acorns, except that No. 1 was relatively smaller on account of the greater thickness of the shell. (Figs. 8 to 11 and 12 to 15.)

The branches and twigs were much alike except that the epidermis of Nos. 1 and 3 split up much earlier than that of Nos. 2 and 4, making the twigs of the former rough while those of the latter were smooth.

The differences enumerated above formed the basis for the microscopic study of the anatomy.

PREPARATION OF MATERIAL.

Material was prepared for sectioning by fixing in Fleming's fixative and embedding in paraffin. Leaves were bleached in chloral hydrate and potassium hydrate. In the study of the wood, sections were made on the hand microtome from material preserved in formalin and softened in dilute glycerine. In tracing the middle bundle of the midrib, formalin-preserved material was used, the sections being made on the hand microtome.

BLEACHING.

There was a notable difference in the length of time it took to bleach the leaves in choloral hydrate. No. 1 bleached in 24 hours, No. 4 in 6 days, Nos. 2 and 3 in 7 days. In potassium hydrate No. 4 was bleached in 24 hours, the others in 48 hours. After bleaching, the leaves were stained 30 minutes in safranin and decolorized in 70 per cent alcohol, which aided greatly in differentiating the various tissues. Examination of the bleached leaves under the microscope immediately revealed the probable cause of the difference in time of bleaching. The walls of the lower epidermis of No. 1 were very thin, almost undistinguishable, while those of the others were relatively thick.

STOMATA.

The size and number of the stomata as revealed in the bleached leaves are features that enable one readily to distinguish between the four oaks. In all the leaves the stomata were more numerous near the midrib than near the edge of the leaf. By the usual method of averaging fields the count of No. 2 ran up to about 2000 per sq. mm. In order to get more definitely and accurately the number of stomata, 1 sq. mm. of leaf surface magnified 134 diameters was projected on paper and each stoma marked. (Figs. 23 to 26.) This showed the following numbers per sq. mm.: No. 1, 1074; No. 2, 1252; No. 3, 912; No. 4, 785. The stomata of No. 3 often occurred in rifts and clumps while those of the other oaks seemed more evenly distributed.

Average length of guard cells: No. 1, .0188 mm.; No. 2, .019 mm.; No. 3, .019 mm.; No. 4, .0189 mm. Average width: No. 1, .005 mm.; No. 2, .0059 mm.; No. 3, .006 mm.; No. 4, .0059 mm. Some of the largest stomata of No. 3 were four times as large as the smallest, a variability immediately noticeable. The others were more uniform in size.

The extreme minuteness of the guard cells and the fact that the stomal opening extends no more than a third of the length of the cells makes the mechanism of the stomata an interesting problem which I think is not fully explained by papers on the subject. The stomata are of the "gramineous" type, but looking down on a surface view of a stoma one sees deep depressions between the guard cells where these extend beyond the ends of the stomatal opening, and the heavy inner walls of the two guard cells facing each other here stand far enough apart to afford considerable room for movement when the cells become turgid (figs. 16 and 19, P and P'). The enlarged lumen at the ends greatly increases the surface of the inner heavy wall subject to the pressure of the cell sap, and these walls being free to move, as just shown, would undoubtedly move toward each other with the increase of turgidity. The points of contact of the two guard cells at the ends of the stomatal opening acting as fulcrums (fig. 16, O), the movement of the thickened walls must exert considerable leverage tending to open the stoma. (Figs. 16 and 18, r and r'.) This mechanism alone would seem to suffice to open the stoma, and it must be one of the most important factors, since a study of the stomata shows that the smaller the stoma the relatively longer are the free-moving walls of the guard cells, so that the leverage is increased. Other parts of the mechanism of the gramineous type of stoma have been explained by other investigators.

In the very young leaf the stomata are not of the gramineous type, but are modified as the leaf matures. In the preserved material of the young leaf the stomata stand open, while they are never found open in the preserved material in the mature leaves. (Figs. 20 to 22.)

LOWER EPIDERMIS.

Aside from the stomata, the lower epidermis showed characteristic differences. As noted above, the cell walls of No. 1 were so thin as readily to distinguish it from the others, and the cells were also much smaller. No. 4 could be easily detected by its relatively large cells with wavy walls, while the walls of the other three were straight. The average area of the lower epidermal cells was as follows: No. 1, .000108 sq. mm.; No. 2, .000142 sq. mm.; No. 3, .000147 sq. mm.; No. 4, .00023 sq. mm. (Figs. 45 to 48, sec. D.)

EPIDERMAL HAIRS.

The number and character of the epidermal hairs are distinguishing features. No. 1 has 55 stellate hairs to the sq. mm., with 5 to 9 branches to a hair. They are small and very thin walled. No. 2 has 70 stellate hairs per sq. mm., with from 4 to 9 branches, and the hairs are generally long and thick walled. No. 3 has 215 stellate hairs per sq. mm., with 6 to 14 branches on each hair. These almost completely cover the surface of the leaf and make immediately distinguishable the smallest section of a leaf. The hairs of No. 3 are generally shorter and thicker walled than in No. 2. No. 4 has 80 hairs per sq. mm., with from 6 to 10 branches on each hair. Except in thickness of wall, they are like those of No. 2, but the walls are much heavier, having the central cavity a mere line. (Figs. 45 to 48, sec. E.)

UPPER EPIDERMIS.

The cells of the upper epidermis of the several oaks differed in size and thickness of walls. No. 1 had the thickest walls. The walls of No. 4 were nearly as heavy, while those of No. 2 were lightest. Comparative average area of upper epidermal cells in sq. mm.: No. 1, .000542; No. 2, .000503; No. 3, .000604; No. 4, .000661. (Figs. 45 to 48, sec. A.)

MESOPHYLL.

In the bleached leaf the palisade cells of No. 2 were seen to be much more numerous and densely crowded together than in the other three oaks. The palisade cells of No. 1 were smaller than the others, but aside from these differences the mesophyll was of no value in classification. The spongy parenchyma was not discernable in the bleached leaf, but tangential sections from paraffin-imbedded material showed that the cells of No. 1 and No. 4 were generally smaller than those of Nos. 2 and 3. These cells, however, varied greatly in the same leaf.

In cross section the palisade cells of No. 2 were seen to vary greatly in length, while those of the other oaks were more uniform. (Figs. 27 to 30.)

VENATION.

Aside from the macroscopic differences already noted, the only constant differences in venation was in the distance apart of the vein endings. These varied in order: No. 2, 1, 3, 4, with No. 4 having the closest venation and No. 2 the most open venation. (Figs. 49 to 52.)

Oaks.	Epidermal hairs.	Stomata per sq. mm.	Lower epidermis.	Upper epidermis	Mesophyll.
No. 1.....	55 per. sq. mm. Very thin walled.	1074 generally uniform.	Average area of cells: .000108 sq. mm. Walls straight, very thin.	Average area of cells: .000542 sq. mm. Walls heavy.	
No. 2.....	70 per. sq. mm. Walls of medium thickness.	1252 generally uniform.	Average area of cells: .000142 sq. mm. Walls straight, of medium thickness.	Average area of cells: .000503 sq. mm. Walls quite thin.	Palisade cells closely compacted.
No. 3.....	215 per. sq. mm. Walls heavy.	912 variable in size and shape.	Average area of cells: .000147 sq. mm. Walls straight, of medium thickness.	Average area of cells: .000604 sq. mm. Walls of medium thickness.	
No. 4.....	80 per. sq. mm. Walls very heavy.	785 generally uniform.	Average area of cells: .00023 sq. mm. Walls wavy, of medium thickness.	Average area of cells: .000661 sq. mm. Walls heavy.	

This summary might serve as a key to classification, and with this idea in view all the oaks with leaves similar to the four types here studied found in the herbarium were examined by means of the bleached leaves. These were from various parts of the United States, and had been given various classifications: *Q. acuminata* (Tennessee), *Q. acuminata* (North Carolina), *Q. acuminata* (Lake Champlain), *Q. prinus* L. (Douglas, Kan.), *Q. prinus* L. (Florida), *Q. prinus discolor* (Illinois), *Q. castenea* Willd. (place of collecting not given).

A microscopic examination showed that those classified as *Q. acuminata* were identical with No. 1 of the four studied. Of the others, *Q. prinus* from Douglas, Kan., *Q. prinus discolor* from Illinois, and *Q. castena,* were identical with No. 3. *Q. prinus* L. from northern Florida was like No. 4 in every particular, with the exception of epidermal hairs, which were entirely lacking. In respect to the large lower epidermal cells with wavy walls, which was the most distinguishing characteristic of No. 4, it was identical. None was found like No. 2.

As a further check on this method of classification, sections of leaves from nine different specimens of *Q. prinoides* found in the herbarium were taken and bleached as before. This oak was selected because of its resemblance to those studied. One of the specimens was from Douglas, Kan., one from Illinois, and the other seven were collected near Troy, Kan., in September, 1913. By the methods used in the study of the four oak under investigation it was found that *prinoides* has 1023 stomata per sq. mm. The epidermal hairs are like those of No. 2, except that they were uniformly much shorter. The palisade cells were closely compacted, as in No. 2, and the cells of the epidermis similar to those of No. 2. The smaller branches of the veins were much more prominent than those of any of the four oaks being studied. These characteristics held good for every specimen of the nine taken, they being so exactly alike that there was no variation that would distinguish one from the others.

This seemed enough evidence to conclude that the wide and constant differences found in the four oaks under consideration were constitutional and not modificational variations.

PETIOLE AND MIDRIB.

Figures 34 to 37 show cross sections of the petioles of the leaves at the base of the leaf blade. They show some differences in the shapes of the petioles and of the various tissues, more especially in the shape of the middle bundle. In Nos. 3 and 4 the bast, phloem, and xylem regions make continuous concentric bands, while in Nos. 1 and 2 the xylem and phloem bands are broken, so that the vascular system is in three bundles. The middle bundles vary greatly in shape, but there is considerable variation in this respect in different petioles from the same individual oak.

Figures 27 to 30 show cross sections of the midrib of the leaves taken at two-thirds up from the base of the blade and a little above the insertion of a vein, which accounts for the bast ring being broken. The sections show that the middle bundle of Nos. 1, 3 and 4 have disappeared, while that of No. 2 still persists.

Cross sections were made on the hand microtome, from material preserved in formalin, to determine the manner and place of the ending of the middle bundle. This method was used as being better than embedded material in keeping the exact position of each section taken. My fellow student, F. W. Mulsow, found that a mixture of safranin and hæmatoxylin makes a very good double strain and greatly shortens the process for temporary mounts. A section can be kept on the point of a needle, dipped in the double strain for a few minutes, washed in seventy per cent alcohol, and mounted in glycerine, with very satisfactory results.

The middle bundle of No. 2 ended a little above two-thirds the length of the leaf, that of No. 4 a little below two-thirds the length, and that of Nos. 1 and 3 a little above one-half the length of the leaf. The size of the leaf and midrib made no difference in the relative distance the middle bundle ascended the midrib. At the point of ending of the middle bundle in a large leaf the midrib might be larger than the midrib at the base of the blade in a smaller leaf.

The branching of the midrib into the lateral veins has an indirect effect upon the middle bundle, as the bundles break up into sections whenever a branch is given off, and in the readjustment a portion of the middle bundle is attached to the upper bundle. The middle bundle does not branch directly into the secondary veins. The termination of the middle bundle in No. 4 is distinctly different from that in the other three oaks, as the middle bundle in No. 4 always disappears by joining to the side of the upper bundle, while the juncture in the other three is in one manner or another always made at the middle of the upper bundle. The manner of joining is essentially the same in Nos. 1, 2, and 3, although in this respect there are variations in leaves of the same tree. (Figs. 31 to 33.)

ACORNS.

The cells of the acorns were approximately the same size, but there was considerable difference in the sizes of the starch grains. The average diameters of the starch grains were as follows: No. 1, .00784; No. 2, .006; No. 3, .00545; No. 4, .00645 millimeters. The cells of the acorns were crowded with starch grains in all cases. (Figs. 38 to 41.)

Iodine, nitric acid and ammonia, and Millon's reagent were used to test for proteins. All three reagents showed more protein in seeds from No. 2 than in the other three, which were about equal in this respect. Ferric chloride indicated about equal amounts of tannin in all, but this was not as abundant in these acorns as in those of most oaks.

Macerations of the shell and cup of the acorns showed a great variety of stone cells and tracheids, but nothing that would distinguish one from the others. (Figs. 42 to 44.)

WOOD AND BARK OF THE CURRENT YEAR.

A cross section of the current year's growth of the stem shows an even gradation in diameter from No. 1 to No. 4. This is mostly due to the differences in thickness of the regions outside of the cambium ring. The growth of the xylem is variable in the same specimen, but the thickness of bark is constant. In No. 1 and No. 3 the cork-cambium produces enough cork in the current year's growth to break up the epidermis, while Nos. 2 and 4 have a smooth epidermis, that of No. 4 persisting for at least three years. The greatest differences were noticeable in the widths of the parenchyma tissues of cortex and pericycle. The bast ring varies in average width as shown in the following measurements: No. 1, .087 mm.; No. 2, .06 mm.; No. 3, .0706 mm.; No. 4, .0435 mm. The average distances between cambium ring and bast ring are: No. 1, .15 mm.; No. 2, .12 mm.; No. 3, .07 mm.; No. 4, .07 mm. The average widths of the parenchyma of cortex are: No. 1, .22 mm.; No. 2, .18 mm.; No. 3, .11 mm.; No. 4, .10 mm. In amount of pith Nos. 1 and 4 were about equal and they had much less than Nos. 2 and 3. The pith cells were about the same size in all. All these variations were immediately noticeable under the microscope. (Figs. 53 to 56.)

OLDER WOOD AND BARK.

In the study of the more mature stem sections were taken from the oldest material at hand. The cross sections showed that the differences in thickness of bark persisted, but that the relative increase in thickness of bark due to the cambium ring was about the same. The original bast ring of No. 2 remained entire for at least seven years. That of No. 4 was unbroken at three years. (Older material not available.) The bast ring of No. 3 was broken up in the four-year-old stem. That of No. 1 was never entire. (Figs. 61 to 64.)

No. 1 showed the slowest growth of xylem and No. 3 the most rapid, from trees very nearly all the same size. Cross sections of the wood showed nothing that would distinguish any one of the oaks except that the medullary rays of Nos. 1 and 2 were wider than those of Nos. 3 and 4. (Figs. 65 to 68.) In tangential section this showed much more clearly and was an identifying characteristic. The sections were taken from growth of the same year. (Figs. 57 to 60.) In the same area (1.04 sq. mm.), the number of medullary rays was as follows: No. 1, 129; No. 2, 63; No. 3, 134; No. 4, 144. The difference between Nos. 1 and 4 was least pronounced. No. 3 could be immediately identified by its very narrow rays, and No. 2 by its large, broad ones.

Macerations of the wood and bark revealed no distinguishing characteristics.

SUMMARY.

The four oaks under consideration, classified as all being *Q. muhlenbergii*, show some difference in general appearance of leaves and acorns. The histology of the leaf reveals constant identifying differences in epidermal hairs, number of stomata, and size of epidermal cells, any one feature of which will distinguish any one individual from all of the others. In addition to these features which are different in each oak, No. 4 is distinguished by wavy-walled cells of the lower epidermis; No. 1 by thin-walled epidermis, and No. 2 by closely packed palisade cells. The manner of ending of the middle vascular bundle of the midrib differentiates No. 4, and the place of ending differentiates No. 2.

There is a difference in the sizes of the starch grains in the seeds. No. 2 is characterized by the presence of the most protein in the seeds.

The four oaks may be distinguished by the thickness of the bark in the current year's growth, and by the tissues of the bark.

There is a distinguishing difference in the number and size of the medullary rays in the wood.

CONCLUSIONS.

No. 1 is the species described as *Q. muhlenbergii* and may be identified macroscopically by its narrow leaves with sharply acuminate, serrate teeth. The shell of the nut is twice as thick in No. 1 as in Nos. 2, 3, and 4. Nos. 2 and 3 are possible hybrid *muhlenbergiis.* They may be distinguished from No. 1 by their broader and more irregular leaves. No. 2 has large acorns, and has a smooth bark for several years, while the epidermis is broken up on Nos. 1 and 3 in the current year's growth. The acorns of No. 3 are very small and the nut is almost overgrown by the cup. A hand lens will reveal the unusual number of epidermal hairs on the lower epidermis of No. 3, which might well give it the variety name of "hirsuta."

No. 4 is enough different from the others to be classed as a distinct species. It may be identified macroscopically by its slightly paler leaves, smooth-barked twigs and a marked difference in the acorn cup, which is shallow, thin, and covered with thin tightly appressed bracts, while those of Nos. 1, 2, and 3 are generally deeper, thicker and covered with thick bracts which make the cups irregular and rough. Either No. 4 or No. 2 is probably *Quercus alexanderi* of Britton and I would suggest that name be retained for No. 4.

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A Comparative Anatomical Study of Some Species of Xanthium.
Nora E. Dalbey.

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A Comparative Anatomical Study of Some Species of *Xanthium*.

BY NORA E. DALBEY.

Plates XVI to XXII.

INTRODUCTION.

EIGHT species of *Xanthium* are now recognized in the territory east of the one hundredth meridian. One species, which in the first edition of the Illustrated Flora of the United States and Canada, by Britton and Brown, was called *Xanthium canadense*, is now known to be two distinct species, *Xanthium americanum* Walter, and *Xanthium pennsylvanicum* Wallroth

In 1912 Prof. Charles A. Shull, of the University of Kansas, for the purpose of securing pure lines for experimental work, made a collection of the seeds of the three main types of *Xanthium* found in the fields about Lawrence. Of the three types, one resembled closely *Xanthium pennsylvanicum*, another agreed well with the description of *Xanthium americanum*, and the third was a form distinguished by short globose burs, to which he gave the provisional name of *Xanthium globosum*. He thinks this type is new, as it has never been described among the published species of *Xanthium*. Professor Shull selected seeds from one plant of each type, and planted them in pots in the laboratory, and about the first of June transferred them to the breeding grounds. It was expected that the plants would show the characteristics of hybrids, but instead, they developed with uniformity, showing three distinct types, and it was from these plants, in the early part of September, 1913, that the material for this study was secured.

LEAF.

There are very distinct external differences between the leaf of *X. globosum* and of *X. pennsylvanicum.*

The leaves of *X. globosum* are dark green and have a much crinkled surface, while those of *X. pennsylvanicum* are lighter in color and have a comparatively even surface. The leaf of *X. americanum* is in its superficial aspect very much like that of *X. globosum.* There are no very striking differences in leaf structure shown in either cross or tangential sections (figs. 1, 2, 3, 4, 9), but some minor differences occur which are constant.

The leaf of each species is petiolate, with about ten to fourteen vascular bundles entering from the stem. The leaf is entered by three large veins, each containing about the same number of vascular bundles (fig. 12). In the apex of the leaf, and in the apex of each deeply cut tooth on the margin, the veins end in clusters of tracheïds (fig. 10, *W*). The midrib and other large veins of the leaf contain a very large amount of thin-walled mesophyll cells, scanty in cell contents (figs. 11, *B;* and 12, *J*). The vascular bundles contain a relatively large amount of phlëm (fig. 21, *X*). The epidermis of the midrib is uniform in shape and size (fig. 15, *N*), with a heavier cuticle layer than on other parts of the leaf (fig. 11). Beneath the epidermis of the midrib there is a layer of collenchyma cells from three to six cells in thickness (fig. 11, *C*).

Rather large border parenchyma cells, which are almost empty of cell contents, surround the vascular bundles of the veins (figs. 1, *A;* 2, *D;* 3, *G*), and extend in plates to the margin above and below (fig. 4, *L*). Resin ducts, in cross section, surrounded by, on the average, six secreting cells, occur in the parenchyma in the midrib and surrounding the larger veins (fig. 12, *G*).

The leaf of each species has the dorsi-ventral differentiation shown in figures 4 and 9. In *X. pennsylvanicum* the palisade tissue has a length in cross section of 3 cells, on the average (fig. 9), while the average in *X. globosum* is 2 cells, and in *X. americanum* 2.5 cells (fig. 4).

The ratio of the area, in cross section, of the palisade tissue to the area of spongy parenchyma is in *X. pennsylvanicum*

1 to 1, and in *X. globosum* and *X. americanum* 1 to 1.6. In *X. pennsylvanicum* the palisade cells of one section varied in length from .033 mm. to .062 mm. (figs. 5 and 6). In the other species this variation was not so great, being .030 mm. to .037 mm. in *X. globosum* (fig. 7), and .030 mm. to .045 mm. in *X. americanum* (fig. 8).

In cross sections of the leaves of the different species, which had been treated with alcannin, the mesophyll cells contained large bodies which stained a bright red. When the sections were put into ether and then treated with alcannin the same bright-red bodies were seen, but when treated to boiling in Fehling's solution, and left in the solution several hours at a temperature of about 35° C., copper oxide crystals replaced these round bodies. Since the reaction for sugar did not occur except after long contact with Fehling's solution, the bodies are probably glucosides, which yield sugar after hydrolysis by long standing in Fehling's solution.

The cells of the epidermis are rather irregular in size (fig. 4, *J*). In cross section the average diameter parallel with the circumference is in *X. pennsylvanicum* .015 mm., in *X. amercanum* .0131 mm., and in *X. globosum* .0135 mm. In tangential section the epidermal cells of *X. pennsylvanicum* are more irregular in shape than the epidermal cells in the other species (figs. 16, 17, 19).

A ridged cuticle layer covers the outer cell walls in each species.

Stomata of the usual type (figs. 14, *M*; 16, *O*) occur on both sides of the leaf, more numerous in *X americanum* than in the other species, as the following tabulation will show:

	Upper side.	Under side.
X. pennsylvanicum	243.5 to sq. mm.	205.8 to sq. mm.
X. americanum	309.9 to sq. mm.	270.5 to sq. mm.
X. globosum	196.0 to sq. mm.	166.0 to sq. mm.

In cross section the guard cells are much smaller than the adjacent epidermal cells, as the following comparison shows:

	Guard cells.	Adjacent epidermal cells.
X. pennsylvanicum	.005 mm. diam.	.017 mm. diam.
X. americanum	.007 mm. diam.	.030 mm. diam.
X. globosum	.005 mm. diam.	.020 mm. diam.

Numerous trichomes of two different forms occur on both surfaces of the leaf (fig. 22, *E* and *F*).

The most conspicuous one is a large hair with an average length of .14 mm., composed of a basal cell, one or sometimes two middle cells, and a long, pointed terminal cell (fig. 24), with the outer wall of all the cells sometimes showing distinct papillæ. Below the basal cell there are a number of very regular cells (figs. 24, *I*; 17, *Q*), larger than the other cells of the epidermis, which in tangential section appear to form a rosette (fig. 17, *Q*). Both forms of hairs are essentially alike in the three species. The number of the large hairs varies in the three species: in *X. pennsylvanicum* 22.4 per sq. mm., in *X. americanum* 53.8, and in *X. globosum* 46.7. The small linear hairs are numerous over the veins. They are composed of from two to eight cells of about equal size (fig. 22, *E*).

The hairs are distributed in definite relation to the veins, for both large and small hairs always occur in connection with the water-storage tissue above and below the veins (fig. 23, *G* and *H*), those above the intersection of two veins being usually larger (fig 23). The cuticle extends unbroken over all the hairs.

STEM.

The young stems are slightly five-angled (figs. 25, 26 and 27), a character which seems to be a little more evident in *X. pennsylvanicum* (fig. 26) than in *X. americanum* and *X. globosum,* while older stems are nearly circular in cross section. At the angles, in the young stems, the parenchyma of the pericycle is not so wide in cross section as elsewhere (figs. 25 to 27). At these angles the vascular bundles are larger (figs. 27 and 28), with xylem in which the rows of vessels show marked radiation (fig. 28). Stems from the three species, of about the same size and age, in cross section show a difference in the comparative amount of each tissue, as indicated in the following tabulation giving radial diameters:

	Collenchyma.	Parenchyma of pericycle.	Phloëm.	Xylem.	Pith.
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
X. pennsylvanicum	.125	.365	.084	.490	4.531
X. americanum	.125	.318	.131	.693	3.876
X. globosum	.156	.375	.156	1.093	3.843

The pith of each species is composed of rather thin-walled, pitted cells (figs. 29, 30 and 31). There is a very slight variation in both longitudinal and transverse diameters of the pith cells of the three species:

	Longitudinal diameter.	Transverse diameter.
X. pennsylvanicum	.110	.070
X. americanum	.132	.071
X. globosum	.118	.076

The cell walls vary in thickness from .0015 mm. to .002 mm., and are cellulose, except those near the wood, which have lignified walls. In each species the average diameter of the pith in number of cells varies from 30 to 35.

Clusters of calcium oxalate crystals, with an average diameter of .015 mm., are regularly distributed throughout the pith (figs. 32, *N*; 33, *O*). The average number per sq. mm. seen in cross section varies in the three species: in *X. pennsylvanicum* 1000, in *X. americanum* 234.5, and in *X. globosum* 493.8.

The pith cells of each species contain a large amount of stored-up material in the form of glucosides. Sections boiled in Fehling's solution did not show a reaction for sugar, but when sealed on slides in Fehling's solution, and kept at a rather high temperature for about forty-five minutes, crystals of copper oxide appeared, indicating the hydrolysis of glucosides.

The wood increases in size by the interpolation of new bundles, the bundles of one section of a growing stem showing a large variation in size (fig. 27). In *X. americanum* and *X. globosum* the ray cells between the bases of the bundles are elongated parallel to the circumference (fig. 41, *N*). *X. pennsylvanicum* does not show this characteristic.

In cross section the vessels are seen in radial rows (figs. 38, 39 and 40), with the rows of vessels in the large bundles at the angles of the stem (fig. 28) separated by a greater amount of wood parenchyma than in other bundles of the same stem (fig. 27). The vessels varied in diameter in a single bundle from .010 to .060 in *X. pennsylvanicum*, .017 to .052 in *X. americanum*, and .015 to .038 in *X. globosum*. The species vary in the per cent of stem area made up of vessels. In *X. pennsylvanicum* and *X. globosum* the water-conducting tissue composes 2 per cent of the stem area, and in *X. americanum* 3 per cent.

Wood parenchyma is scanty in amount (figs. 38, 39, 40), and the stem is made strong by a large amount of lignified wood fibers (fig, 42, *P*). In *X. pennsylvanicum* 90.5 per cent of the xylem is lignified, in *X. americanum* 85.1 per cent, and in *X. globosum* 86.7 per cent.

The wood fibers average in diameter .0113 mm. in *X. pennsylvanicum*, and .0110 mm. in *X. americanum* and in *X. globosum*.

The wood parenchyma also contains glucosides and calcium oxalate crystals.

The cambium cells are not always discernible, and do not form a complete ring.

The phloëm is made up of a mass of very irregular thin-walled cells (fig. 34), which are not differentiated into sieve tubes and companion cells. These thin-walled cells vary in diameter from .004 mm. to .020 mm. in *X. pennsylvanicum,* from .005 mm. to .022 mm. in *X. americanum,* and from .005 to .020 mm. in *X. globosum.*

The phloëm is rich in cell centents, containing glucosides, a rather small amount of protein, and numerous bodies which give the mucilage stain with methylene blue.

In each species a starch sheath is discernible (figs. 35, 36, 37), the cells containing very large crystals of calcium oxalate (fig. 35, *P*) and rather large starch grains (fig. 36, *T*), which are more numerous in *X. americanum* than in the other species. In transverse section the cells of the starch sheath have an average diameter of .0357 mm. in *X. pennsylvanicum,* .0270 mm. in *X. americanum,* and .0261 mm. in *X. globosum.*

The medullary rays vary in width from 3 to 9 cells in *X. pennsylvanicum,* from 2 to 6 in *X. americanum,* and from 2 to 5 in *X. globosum,* with an average width in number of cells of 4.5 for *X. pennsylvanicum,* 2.5 for *X. americanum,* and 3.4 for *X. globosum.* The ray cells of the xylem are lignified and have pitted walls. They have an average radial diameter of .0254 mm. in *X. pennsylvanicum,* of .024 mm. in *X. americanum,* and of .027 mm. in *X. globosum.*

There is a striking variation in the size and shape of the different bast groups seen in one cross section of a single species (fig. 26).

In one cross section the bast masses varied in size from a mass .045 mm. in diameter composed of only six cells, to a mass .570 mm. in diameter made up of many cells. All bast cells are lignified with strongly pitted walls .004 to .005 mm. in thickness.

The parenchyma of the pericycle is composed of large thin-walled cells and contains numerous air spaces (fig. 49). Here frequent resin ducts occur (fig. 49, *T*) surrounded by, on the average, six very small secreting cells (fig. 50, *X*), which are rich in cell contents. They contain a very small amount of protein, small masses which stain a deep blue in methylene blue, and when sections were treated with Sudan III bodies were found in these cells which seemed to be oil.

The parenchyma of the pericycle contains glucosides, calcium oxalate crystals (fig. 53), and numerous oval bodies (fig. 52) which stain for mucilage with methylene blue, and give the characteristic red color with alcannin and Sudan III.

The collenchyma, in cross section from 4 to 6 cells deep (figs 49, *R;* 50, *V*), is composed of comparatively small thick-walled cells with minute air spaces at the corners, which are rather more numerous in *X. pennsylvanicum* than in *X. americanum* or *X. globosum.* In each species the subepidermal layer (figs. 49 and 50) resembles the epidermis and is richer in cell contents than other cells of the collenchyma.

In cross section (figs. 57 and 58) the diameter of the epidermal cells parallel with the circumference of the stem is on the average .0197 mm. in *X. pennsylvanicum,* .0140 mm. in *X. americanum,* and .0190 mm. in *X. globosum.* In longitudinal section the diameter averages .0428 mm. in *X. pennsylvanicum,* .0516 mm. in *X. americanum,* and .0460 mm. in *X. globosum.* The epidermal cells of each species contain numerous glucoside bodies. In tangential section they appear either in the form of single droplets or masses (figs. 55, *D;* 56, *F*), or in the form of clusters of minute droplets.

Slight cutinization occurs in the outer walls of the epidermal cells. *X. pennsylvanicum* and *X. globosum* have a ridged cuticle .0015 mm. in thickness, while the cuticle in *X. americanum* is of the same thickness but less ridged.

Two forms of hairs similar to those found on the leaf occur on the stem (fig. 54). In *X. pennslyvanicum* both forms are very scanty, while in *X. americanum* there are on the average 2.57 large and 4 small linear hairs to the sq. mm., and in *X. globosum* 1.17 large and 1 small linear hair to the sq. mm.

ROOT.

The cross section of the tap root shows a protostele figs. 60, 61) with vessels varying in diameter from .025 to .135 mm. in *X. pennsylvanicum,* from .025 to .150 mm. in *X. americanum,* and from .015 to .120 in *X. globosum.* In cross section the ray cells are generally composed of three rows of cells, the central row made up of ray tracheids with bordered pits. All cells of the xylem have lignified walls.

The phloëm varies in thickness in the three species. In *X. pennsylvanicum* it is .150 mm., in *X. americanum* .202 mm., and in *X. globosum* .195 mm. in thickness.

In *X. pennsylvanicum* and *X. globosum* the phloëm at two opposite sides of the root is thicker than at other parts of the root (figs. 60 and 61), and extends into the xylem. *X. americanum* does not have this characteristic. The phloëm in each species has irregular areas in which the cell walls are lignified.

SUMMARY.

1. The three types of *Xanthium* described present some striking external characteristics, while in their anatomy there are some definite but minor differences which might prove of uncertain value in classification.
2. In each species three large veins enter the leaf, the midrib and other large veins containing a very large amount of thin-walled parenchyma cells in which resin ducts occur.
3. The leaf of *Xanthium pennsylvanicum* has a little more palisade tissue than the leaf of *Xanthium americanum* and *Xanthium globosum.*
4. The stomata are more numerous in *X. americanum* than in *X. pennsylvanicum* or *X. globosum.*
5. Numerous trichomes of two different forms occur on both surfaces of the leaf. The most conspicuous is relatively large, awl-shaped, and composed of three cells; the other form is linear. These always occur in connection with the water-storage cells above and below the veins.
6. A cuticle layer covers the entire outer wall of the hairs in each species.
7. The young stems are slightly five-angled in outline, with *X. globosum* containing a little more wood than the other two species described.
8. *X. americanum* and *X. globosum* have the ray cells between the bases of the bundles elongated tangentially. *X. pennsylvanicum* does not show this characteristic.
9. *X. americanum* has 3 per cent of the stem area given to vessels; *X. pennsylvanicum* and *X. globosum* have 2 per cent.
10. The phloëm is not differentiated into sieve tubes and companion cells.
11. In each species resin ducts occur in the parenchyma of the pericycle.

12. The starch sheath is discernible in each species, and contains starch grains and calcium oxalate crystals.

13. The epidermis of each species contains glucosides in the form of single droplets or of clusters of droplets.

14. Each species has a ridged cuticle.

15. Hairs similar to those found on the leaf occur on the stem, numerous in *X. globosum* and *X. americanum,* but scanty in *X. pennsylvanicum.*

16. Glucosides occur in each species in the phloëm, the parenchyma, the wood parenchyma and the pith.

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CONTENTS:

On Cubic Surfaces and Their Nodes, ... *S. Lefschetz,*

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On Cubic Surfaces and Their Nodes.

BY S. LEFSCHETZ.

1. The present paper is chiefly concerned with the development of a construction of non-ruled cubic surfaces given by R. Sturm(¹) It is first shown rapidly how from this construction the whole theory of the surface can be obtained by applying the principle of correspondence, then the modifications necessary to obtain the 21 different species are given. While very few of the results obtained are new, the general treatment is, as well as the use of synthetic methods to obtain constructions of nodal cubic surfaces. Furthermore, these processes are susceptible of extension to surfaces of higher order, but we reserve this for a later occasion.

2. Before entering upon our main subject we will treat the following problem: *To find the number of common bisecants to two arbitrary twisted curves.*(²) Let R_m, R_n be the two curves, m, n their order, δ_m, δ_n the number of double edges of their projecting cone from an arbitrary point in space. It will be sufficient for our purpose to consider the case where three of the common bisecants are trisecants for either curve. Let then A be a point on R_m, from which we draw one of the bisecants of R_n that go through it, and meet R_n in a' and a''. Through either of the latter points draw one of the bisecants of R_m, which will meet it in 2 points, one of which we will call B. To every point A there correspond $2\delta_n$ points a, and therefore $4\delta_m\delta_n$ points B. Also to every point B there correspond all the points in which R_n meets the cone of order $(m-1)$ projecting R_m, from B, and they are $n(m-1)$in number. Similarly to every

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1. Flachen dritter Ordnung. p. 338.

2. Another solution given in Basset Geometry of Surfaces. p. 230.

point a there correspond $m(n-1)$ points A, hence to B there correspond $m\,n(m-1)\,(n-1)$ points A. It follows that A and B are in a correspondence $[4\delta_m\,\delta_n,\ m\,n(m-1)\,(n-1)]$, and since if A is arbitrary on R_m, one of the points B coincides with it, the number of coincidences is $[4\delta_m\,\delta_n+m\,n(m-1)\,(n-1)]$. It is seen at once that when A coincides with B, $A\,a$ is a common bisecant, and further that if A' is the other end of the latter, then two points B coincide with A, and two of them with A', so that to a common bisecant there corresponds four coincidences in the above correspondence, and therefore the number of common bisecants is

$$\delta_m\,\delta_n+\frac{mn\,(m-1)\,(n-1)}{4}$$

In particular suppose $m=n=3$, then we know that $\delta_m=\delta_n=1$, and therefore

$$\delta_m\,\delta_n+\frac{mn\,(m-1)\,(n-1)}{4}=10.$$

In other words *two twisted cubics have ten common bisecants.*

3. Let now R_1^3 and R_2^3 be two twisted cubics having a common point O. They still have ten common bisecants. For the only thing that could change this circumstance, would be multiple coincidences in O, and that this is not the case can be shown very simply by considering two twisted cubics having five common points, and ten common bisecants, namely the lines joining these points two by two. Now the quadric cones projecting the two curves from O have four generators in common which are common bisecants, and therefore: *When two twisted cubics go through the same point, they have six common bisecants not going through this point.*(3) We will denote these lines by $a_1, \ldots\ldots\ldots a_6$. Let now P be an arbitrary plane through O, OD any line in P, Σ_1, and Σ_2 the two quadrics defined respectively by R_1^3, and OD, R_2^3 and OD. Their intersection is composed of OD and a twisted cubic r^3. When OD describes the plane P, the two quadrics describe two projective pencils of quadrics, and therefore their intersection has for its locus a quartic surface. But P is a part of the latter, hence the locus of r^3 is a cubic surface S_3, which will contain R_2^3, R_1^3 and the lines $a_1 \ldots\ldots a_6$ as having four points in common with each of them. This surface S_3 goes through a fixed curve of order $3+3+6=12$, when P varies, and therefore is absolutely independent of the plane P. Hence *two twisted cubics that meet in a point define one and only one cubic surface.*

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3. R. Sturm. Die Lehre von den Geometrischen Verwandschaften. Vol. II, p. 188.

4. We will now proceed to show rapidly how the fundamental properties of the surface can be deduced from the preceding generation. If $i \neq k$ then a_i and a_k have no point in common, since they are bisecants of a twisted cubic. No quadric can contain four of the lines a, for all its generators of the other system would then be on S_3, which would then decompose. Let Σ be the quadric defined by a_1, a_2, a_3. A plane through a_1, is tangent to S_3 in the two points where a_3 is met by its residual conic, and to Σ in one point. The points of contact of such a plane with the two surfaces are therefore in a (1, 2) correspondence, and its 3 coincidences show us, that the residual intersection of Σ and S_3 is formed of three straight lines. On the other hand there are two generators of Σ that meet a_4, and similarly for a_5 and a_6, while such generators are common to S_3 and Σ, as having four points on S. The only way to satisfy these two conditions, and those obtained by a cyclic substitution on the a's, is to have six lines $b_1, \ldots\ldots\ldots\ldots b_6$, such that each meets all the lines a, of different index. We have thus obtained the double six of Schlaefli.(4) The plane $a_i b_k$ meets S_3 in a third line c_{ik}, while it meets a_k in a point that must be on c_{ik}, since it is neither on a_i nor b_k. We have thus 15 new lines c_{ik} $(i=1, \ldots\ldots 6,\ k=1, \ldots\ldots 6)$, since c_{ik} coincides with c_{ki}, and therefore the 27 lines of the surface. Since a_i meets b_k it does not meet c_{ik}, which therefore cuts the plane $a_i b_l$ in a point necessarily on c_{il}.

The cubics r^3 do not cut a_i, for the latter meets Σ, in two points on R_1^3, and therefore nowhere else. But b_i cuts Σ_1 in two points which are not on R_1^3, or else, being a bisecant of R_1^3 it could not meet a_k, and therefore these points are on r^3. Finally the plane $(a_i b_k c_{ik})$ cuts R_1^3 in three points on S^3, and since one is on b_k, and only two on a_i, the third must be on c_{ik}, which therefore meets R_1^3, and as well R_2^3. Then R_1^3 cuts Σ_2 in six points one of which is O, therefore it meets r^3 in five points. The curves r^3 form a linear series on S_3, which is ∞^2 like OD. Through any point of the surface there go ∞^1 such curves, and since r^3 is residual of R_1^3 on δ_3 their relation is entirely reciprocal, and shows the existence of two sets of ∞^2 twisted cubics on S^3 corresponding to the double six $(a_i)(b_k)$. The system (R_1^3, R_2^3) depends upon 23 arbitraries, since each one of these curves is determined by six points, and that O is variable on one of them. On the other hand O is arbitrary on S_3, and through it there go ∞^1 curves R^3, hence S_3 depends upon $23-2-2.1=19$ arbitraries.(5) If the absolute invariants are taken as parameters,

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4. Quarterly Journal. Vol. II, p. 115.

5. R. Sturm. Die Lehre von den Geom. Verwandschaften. p. 193.

and if we consider only classes with respect to the general projective group, we can say that there are only $\infty^{19-15} = \infty^4$ classes of S_3.

5. (6)Suppose now that R_1^3 and R_2^3 have, besides O, another common point D. Let DT_1 and DT_2 be their tangents in D. Then Σ_1 and Σ_2 pass both through D, which will be a fixed point of the curve r^3 generating the surface. The tangent planes to the two quadrics in D_1 pass respectively through DT_1 and DT_2, and are projectively related. Therefore their intersection, which is tangent to r^3 in D, describes a quadricone. Hence D is a conic node of the surface. Two twisted cubics cannot have more than five points in common, since only one goes through six points. *Hence a cubic surface has at most four nodes.* The construction of surfaces with 1 , 2. 3, 4 nodes follows at once.

If the surface has only one node say D, three of the lines a go through D, and are obtained by projecting R_1^3 and R_2^3 from D, and taking the three common generatrices of the projecting cones other than OD. Let a_1, a_2, a_3 be these lines, then a_4, a_5, a_6 are the three remaining common bisecants or : *Two twisted cubics having two points in common have three common bisecants not going through either common point.* It follows readily that three of the lines b pass through D, and also that all these lines through D are binary.

Let now the *surface have two nodes, D_1 and D_2.* Then it is seen at once that of the lines a, for example a_1 coincides with D_1D_2, a_2 and a_3 go through D_1, a_4 and a_5 through D_2, hence only a_6 is left as common bisecant of R_1^3 and R_2^3, going through one of their common points. But b_6 must meet $a_1 \ldots\ldots a_5$, hence it coincides with a_1, and b_1 meets $a_2 \ldots\ldots\ldots\ldots a_6$, hence it is the intersection of the planes (a_2, a_3) and (a_4, a_5) and meets a_6. Hence this proposition: *When two twisted cubics have three common points they have one and only one common bisecant going through none of them. Their* 6 *common bisecants going through only one of the common points form three planes that meet on the preceding bisecant.* D_1D_2 which is called the *axis* is easily seen to be a *quaternary line,* the 4 other lines through either nodes are binary, so that there are $27-4-4.2.2=7$ *unary* or simple lines.

Nothing particular can be said about surfaces with three or four nodes, except that their properties are readily deduced from our construction, but it is not worth while entering into any details.

6. Suppose now that the plane T_1DT_2 corresponds to itself, in the projectivity between the tangent planes of Σ_1 and Σ_2 in D. Then the tangent cone in D decomposes into the plane T_1DT_2 and another,

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6. For the surfaces with nodes, we will follow the usual relation. See Salmon, Geometry of Three Dimensions, 4th ed., p. 489.

and we obtain a binode B_3. This will happen when for a certain position, Σ_1 and Σ_2 are tangent in D. If O_3 is their common generator through O, cutting their common tangent plane T_1DT_2 in Q, then DQ is on both quadrics and therefore on S_3. Furthermore on either Σ_1 or Σ_2, DQ is of the other system than OQ, hence it is a bisecant of R_1^3, and of R_2^3 as well. Conversely if DQ common bisecant of R_1^3 and R_2^3, is in the plane T_1DT_2, and if Q is any point on DQ, then DQ is on both quadrics and therefore on S_3. Furthemore on either Σ_1 or Σ_2, DQ is of the other system than OQ, hence it is a bisecant of R_1^3, and of R_2^3 as well. Conversely if DQ common bisecant of R_1^3 and R_2^3, is in the plane T_1DT_2, and if Q is any point on DQ, then there exists a surface Σ_1 going through OQ, and it will contain DQ, since it meets it in three points. The same will be true of Σ_2, and therefore these two surfaces will be both tangent in D to the plane T_1DT_2 which will correspond to itself in the projectivity of the tangent planes. Hence: *the necessary and sufficient condition for* D *to be a biplanar node of* S_3, *is that the plane of the tangents of* R_1^3 *and* R_2^3 *in* D *contain one of their common bisecants through* D.

Therefore if we propose to construct a cubic surface having *a biplanar node* we can give ourselves three coplanar and collinear lines DT_1, DQ, DT_2, two points M_1 and M_2 on DQ, and an arbitrary point O. R_1^3 will then be subjected to the condition of passing through O, D, M_1, and be tangent to DT_1, and R_2^3 to that of passing through O, D, M_2 and be tangent to DT_2. This offers no difficulty whatsoever. In particular if M_1 is made to coincide with M_2 *then we have a surface* (B_3+C_2) that is having a conic node and a binode. By making both R_1^3 and R_2^3 pass through a fourth point quite arbitrary, the construction is still possible and we *have the surface* (B_3+2C_2) having two conic nodes and a binode.

Consider now the points O, D_1, D_2. Through D_1 draw two lines $D_1T'_1$, and $D_1T'_2$ such that their plane contain D_1D_2, then through D_2 two arbitrary lines D_2T_1'', D_2T_2'' and in their plane a line D_2Q on which we will take two arbitrary points M_1 and M_2. We know how to construct a twisted cubic through O, D_1, D_2, M_1 and tangent to D_1T_1'', and D_2T_1''—it is only a special case of the construction of a twisted cubic through 6 points—let it be R_1^3. Similarly we can construct R_2^3 through $OD_1D_2M_2$, and tangent to D_2T_2' and D_2T_2''. *The two curves define a surface* $(2B_3)$ that is with two binodes. If now M_1 and M_2 coincide, which does not affect the construction of the surface, *we have the case* $(2B_3+C_2)$, or a surface with two binodes and one node.

Let us now try to construct a *cubic surface having three binodes* that is the surface $(3B_3)$. Consider a tetrahedron $SABC$, and draw the lines AT_1', AT_2'' in the plane SAB, BT_1'', in the plane SBC, CT_1''' in the plane SCA. We can construct a twisted cubic R_1^3 going through A, B, C and tangent to AT_1', BT_1'', CT_1'''. If we take then a point O arbitrarily on R_1^3 we may remark that R_2^3 is perfectly determined by the conditions that it pass through O, A, B, C, be tangent to AT_2' and to the planes SBC and SCA in B and C. For let E be the point where AT_2' meets SB. Then the conic projection of R_2^3 on the plane SBC must go through ω, $A\ B, C, E$ and be tangent to SC, ω being the projection of O on the plane SBC, while the conic projection of R_2^3 on the plane SAB must go through A, B, $\omega'$and be tangent to SB and AE. R_2^3 is thus perfectly determined as the intersection of the projecting cones from two of its points.

7. Suppose now that R_1^3 and R_2^3 be tangent in D, and let DT be their common tangent. Then the two projective pencils of the tangent planes of Σ_1 and Σ_2, have DT for common axis. The double planes of the projectivity in question will be the tangent planes to S_3 in D, and their intersection DT will lie on the surface. *D will therefore be a binode B_4* that is such that the intersection of the biplanes be on the surface. This is all clearly shown when the contact of the two curves is considered as the limit of two common points infinitely near each other, and this consideration shows also that the point B_4 is equivalent—as far as the class of the surface goes—to two ordinary nodes. There is no difficulty therefore in constructing the surface B_4, having a binode in D. We give ourselves DT and O, and have to construct two twisted cubics going through O, D and tangent to DT. By subjecting the two curves to the condition of having one or two more common points, which can be chosen arbitrarily we obtain the surfaces $(C_2 + B_4)$ and $(2C_2 + B_4)$.

We can easily obtain the *lines on the surface B_4* by direct considerations(7). There are two common bisecants of R_1^3 and R_2^3 through D, each of them counting for two, since there is contact of the two curves in D, while DT is also on S_3. We thus have $a_1, \ldots\ldots\ldots a_5$, therefore R_1^3 and R_2^3 have a bisecant a_6 not going through D. Suppose that $a_2 \equiv a_3$, $a_4 \equiv a_5$. Then the two biplanes are (DT, a_2) and (DT, a_4), and if they are met by a_6 in E and F, the lines DE and DF are also on S_3. Let now P be an arbitrary plane through DT, it meets R_1^3, R_2^3 and a_6 in points G_1, G_2 and H, and HG_2 meet respectively DT' in points K_1,K_2, which are seen at once to be in

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7. Cailey, A Memoir on Cubic Surfaces. Phil. Transacts. 1869. p. 231. Schlaefli, Ibid. 1863. p. 193.

(1, 1) correspondence, and the two coincidences of the latter show that there are two lines meeting R_1^3, R_2^3, DT and a_6. Of these one goes through O, and the other a *transversal* t meets the surface S_3 in four points and therefore belongs to it. The plane (DE, a_4) meets R_1^3 and R_2^3 in the two points not on a_2 and defining a line e on S_3. Similarly the plane (DF, a_2) defines a line f on S_3. both these lines meeting t in the points where it cuts the planes (DE, a_4) (DF, a_2) respectively. Finally the plane (a_2, a_4) meets a_6 and t in two points defining a third line g. This system of lines is identical with that found by Schlaefli as we should expect.

8. Consider now the three points O, D_1, D_2, and three coplanar lines D_2T_1, D_2T_2 and $D_2M_1M_2$. We could define an S_3 having a C_2 in D_1 and a B_3 in D_2 by considering the twisted cubics R_1^3 and R_2^3, the first passing through OD_1 D_2M_1 and tangent to D_2T_1, and the second O, D_2, D_2 M_2 and tangent to D_2T_2. We wish to find *what will happen when D_2 is made to approach D_1 indefinitely.* If we consider the two conics Γ_1 and Γ_2 going through D_1 and D_2 and tangent to R_1^3 and R_2^3 respectively, in D_2, there are ∞^1 quadrics going through them, all tangent to the plane $T_1D_2T_2$ in D_2. At the limit Γ_1 and Γ_2 will osculate R_1^3 and R_2^3, and the plane tangent to the quadrics containing both of them will contain a common bisecant of R_1^3 and R_2^3 through D. We can also say that the tangent plane in D_1 to any quadric osculating both R_1^3 and R_2^3 at that point contains a common bisecant. *Hence the following construction.* Let DT be a tangent to a given quadric Σ, through DT consider two planes cutting Σ in the conics Γ_1 and Γ_2, and in the plane P tangent to Σ in D take two points M_1, M_2 collinear with D. If O is an arbitrary point in space, R_1^3 will go through O, D, M_1, and osculate Γ_1 in D, while R_2^3 will go through O, D, M_2 and osculate Γ_2 in D. The construction presents no difficulty—R_1^3 for example is determined by its two projecting cones from D and O, the first being tangent to the plane of Γ_1 along DT and going through M_1 and O, while the second osculates Γ_1 in D and goes also through M_1. *We thus have the surface with a binode B_5* equivalent to $(C_2 + B_3)$.

In particular suppose that the planes of Γ_1 and Γ_2 coincide, or that R_1^3 and R_2^3 have the same osculating plane, in D. Then M_1 and M_2 will be the points infinitely near D on the respective cubics, and can still be considered as being collinear with D. It is clear that the preceding case is obtained as the limit of the one in which D_1 is a binode B_3 as well as D_2, for then D_1D_2 is in the plane of the tangents to the curves in D_1, and the latter becomes therefore a common osculating plane at the limit. *This construction will then give the binode* B_6 equivalent to $2B_3$.

If in the two preceding constructions the two cubics were subjected to pass through another fixed point D_3 we would obtain *the surfaces* $(C_2 + B_5)$ *and* $(C_2 + B_6)$.

The foregoing conclusions are easily verified by analysis. Let us take as an example the case of B_5. This surface has for equation([8]):

$$WXZ + Y^2Z + YX^2 - X^3 = 0 \qquad (1)$$

If we consider the following ∞^2 system of quadrics:

$$XY + (lX + mZ)(Y + Z) = 0 \qquad (2)$$

$$(X - W)(lX + mZ) - (Y - Z)Z - XW = 0 \qquad (3)$$

it represents ∞^2 twisted cubics on (1), for if we eliminate $(lX + mZ)$ between (2) and (3) we obtain (1), while (2) and (3) have in common $X = Z = 0$. (2) represents the projection of R^3 general cubic of the system on $W = 0$, and as R^3 is tangent to $X = Z = 0$ at the point (0, 0, 0, 1), the osculating plane at the latter has for equation $X + (lX + mZ) = 0$. From (3) we deduce that it meets R^3 on the quadric. $X^2 + Z(Y - Z) = 0$ which is a cone tangent to $Z = 0$, in which $Y = Z = 0$ fixed bisecant of R^3 through (0, 0, 0, 1) or D is situated. Any two curves R_1^3 and R_2^3 of the system considered satisfy therefore the condition that they are tangent in a point D, and that one of their common bisecants through D touches a quadric osculating them at that same point, as we found above.

We may remark that the plane P touching in D the quadric osculating R_1^3 and R_2^3 is evidently one of the biplanes.

9. If in the construction for S_3 with a binode B_4, the cones projecting R_1^3 and R_2^3 from the latter are tangent we obtain a binode in which the biplanes coincide that is a *U-node* U_6. For if D is the binode, DT the tangent to both curves, the biplanes are as we have seen, the planes going through DT and the two common bisecants of R_1^3 and R_2^3 through D, different from OD, and they both coincide in the case considered with a_2. R_1^3 and R_2^3 still have a bisecant a_6 going through neither D nor O, and there is still a transversal t as for B_4. If a_6 meets the U-plane in E, DE is a line on S_3, and finally the plane (a_6, t) meets the plane tangent to the projecting cones of R_1^3 and R_2^3 along a_2 in a line g on the surface. This last result is obtained by considering the limiting position of the plane (a_2, a_4) when a_4 approaches indefinitely a_2. We thus have

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[8] Basset Ibid. p. 61.

obtained for U_6 directly the well known system of lines existing on the S_3 having such a point. But it will be well to verify our conclusions by analysis, for we have shown here that U_6 on a cubic surface is not equivalent to $3C_2$, but is a special singularity.

If

$$W(X+Y+Z)^2+XYZ=0 \tag{1}$$

is the equation of an S_3 having an U_6 in (0, 0, 0, 1), then it is seen at once that:

$$X[h(X+Y+Z)-kY]-(X+Y+Z)^2=0 \tag{2}$$

$$W[h(X+Y+Z)-kY]+YZ=0 \tag{3}$$

represent ∞^2 twisted cubics R^3 on S_3. The cone projecting them from the U-node are prepresented by (2) and they are tangent to $X=0$, where it is met by $X+Y+Z=0$. These R^3 meet $X+Y+Z=0$ and $Z=kW$ the first being a point of contact as can easily be seen. The line $X=W=0$ is effectively on the surface, and it is the intersection of the fixed tangent plane $X=0$ to the cones (2), with the plane $W=0$ which is the plane containing $W=Y=0$, and $W=Z=0$, the only other two lines of the surface, not going through the node. Our conclusions obtained by direct considerations are thus verified.

10. If in the preceding construction for U_6, R_1^3 and R_2^3 have the same osculating plane in D, and still the same tangent $D\,T$at that point, then a_2 will coincide with DT and *we will have obtained a surface with a node* U_7, such that the U-plane contains only two lines, while the only other line on the surface will be a_6, which meets the common osculating plane in a point E, situated on DE, the second line of the U-plane. We will not carry out the analytical verification, which is as easy as before.

The surface with a node U_8 is the only one which cannot be obtained by the method used so far. The reason of it, *is that this surface*, as we shall prove now, *contains no twisted cubics.* For as is well known, there is only one straight line D on such a surface, and it is obtained by the coincidence of the 27 lines of the ordinary one. Hence a twisted cubic R^3 on our surface must meet D in two points. Now there are ∞^1 quadrics going through R^3 and D, and they must meet S_3 in ∞^1 conics. Anyone of the latter has only D for residual intersection, and is therefore necessarily in a plane going through D, the latter meeting the corresponding quadric in a line of the third order, is part of it. Hence the ∞^1 quadrics considered are necessarily decomposed into two planes, which shows that R^3 on a cubic surface with U_8 is necessarily a plane curve. We will give below a construction for this surface.

11. It has been shown by Steiner(9) that when a pencil of planes and a pencil of quadrics are projectively related, the intersection of the corresponding surfaces generates a cubic surface. It can be proved as in §5 that when the bases of the two pencils have a common point, the latter is a node of the S_3. We will not treat all the possible cases this way, as we doubt if the generality that we have reached can thus be obtained easily. Let us consider only the case where the axis of the quadrics is composed of two conics, having then necessarily two common points D and D', while the axis DE of the pencil of planes goes through D, and is in T fixed tangent plane of the pencil of quadrics in D. If Q_1 and Q_2 are the planes of the two conics, they form together one of the quadrics of the pencil, to which will correspond a plane P in the pencil of planes. The lines (P, Q_1) and (P, Q_2) are on S_3.

Further if Σ is the quadric corresponding to the plane P in the plane-pencil, then the two lines l_1 and l_2 of this quadric in P are also on S_3. It follows that since DE is also on S_3, the latter has in D a binode, since the lines on the surface through D, are in two planes through DE, which is then the edge of the binode. The latter is clearly a binode B_4, since its edge DE is on S_3.

Suppose now that T corresponds in the plane-pencil to the system (Q_1, Q_2) in the pencil of quadrics. *There* $T \equiv P$ *and the surface has in* D *a point* U_6. The three lines on S_3 through D are DE, (T, Q_1) and (T, Q_2). Hence if in the same conditions DE coincides with (T, Q_1) that is, is tangent to one of the conics in D, *we have the surface with a node* U_7, and if finally the two conics be both tangent to DE in D, then *we have a surface with a node* U_8, the U-plane of which will be the plane tangent to the quadrics osculating both conics in D, or, if we prefer the plane tangent in D to a cone going through both given conics. We thus have a gometric construction for the surface U_8.

Analytically the general equation of an S_3 with a node is

$$WX^2 + X\phi_2(y.z) + \phi_3(Y\,{}^1Z) = 0$$

which can be generated by the system:

$$WX + \phi_2 - \lambda\phi'_2 = 0 \qquad (1)$$

$$\lambda X = aY + bZ, \text{ if } \phi_3 \equiv (Y + bZ)\phi'_2, \qquad (2)$$

which shows that the axis of the plane-pencil $X = O$, $aY + bZ = 0$ is in $X = 0$, fixed tangent *plane of the pencil of quadrics, and goes through its contact* (0, 0, 0, 1).

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9. R. Sturm. Flachen Dritter Ordnung. p. 9.

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A Contribution to the Subject of the Factors Concerned in Soil Productivity.

CONRAD HOFFMANN,

Secretary of the University of Kansas Y. M. C. A.*

Plates XXIII to XXVII.

THE question as to the presence or absence in certain soils of toxic substances which exert a detrimental effect upon the growing crops is a matter of considerable importance. Ever since the time of de Candolle[1] there has existed a more or less extensive belief that crops excrete substances from their roots which are of a toxic nature. He it was who first attempted to explain certain phenomena associated with systems of crop rotation on the basis of plant-root excretions. He asserted that plants excrete from their roots substances that remain in the soil and injure other plants of the same species but do not necessarily affect plants of other species. In a crop rotation these toxic substances are supposedly destroyed during the period the other species of plants are grown, and so are removed before the original crop is again grown. In case, however, the same crop is continuously grown, these substances not only persist but accumulate, and sooner or later exert a detrimental effect with a resulting diminution in crop yields. In this way de Candolle thus early endeavored to ac-

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* Experimental work for this article was done in the laboratories of the Department of Agricultural Bacteriology of the College of Agriculture, University of Wisconsin, Madison, Wis.

[1] De Candolle, Physiologie Vegetale, 1832, T. 111, p. 1474.

count for the better crop yields secured by rotation than by continuous one-cropping.

This explanation of de Candolle's has been largely retained up to the present time. As evidence of this one finds the following statement in a recent bulletin of the Bureau of Soils:[2]

"We must regard the excreta of growing roots as one of the main causes of the low crop yields obtained in improper crop rotation."

In other words, the toxicity which apparently exists in certain soils is thus attributed in large measure to the root excretions of plants. The work of Czapek and others, however, seems to indicate but little excretive activity on the part of plant roots. One is thus forced to look elsewhere for an explanation as to the source of these so-called toxins, if such exist in the soil.

A more frequent explanation of the diminishing crop yields is that the successive crops remove plant food, so that the subsequent crops have less available food and hence are obviously unable to produce such large yields. In connection with this latter explanation it is well to mention the investigations of Cameron, Whitney, Schreiner, Livingston[3] and others. Their work has been largely responsible for focusing research on the subject of soil toxicity as a cause of reduced soil productivity. According to them the reduced yields are not due to a lack of plant food, but rather to the presence of certain harmful substances in the soil solution. They believe that the concentration of the soil solution is practically the same in all soils.

Evidence both disproving and substantiating the statements of the above investigators relative to the presence of such soil toxins has been secured. It is an established fact that a deleterious effect upon crops is frequently exerted by an excess of certain mineral compounds. Of the latter, salts of magnesia, iron pyrites and sodium chloride are most frequently associated with such harmful effects exerted by soils on growing crops. The presence of excessive amounts of nitrates is likewise frequently responsible for such inhibitive action. This fact is well demonstrated by the work of Headden and Sackett.[4] They have shown that in certain localities of Colorado accumulations of nitrates have occurred which have resulted in a complete cessation of plant growth. The presence of various organic compounds has also been demonstrated to exert a harmful, if not a toxic action, at least when these substances are dissolved in the soil extracts. Schreiner[5] thus attributes to di-hydroxystearic acid a decided toxic property. He states that

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[2] Bulletin 40, Bureau of Soils, U. S. Dept. Agriculture, p. 40.

[3] Bulletins 22, 23, 28, 30, 36, 40, 47, 80, Bureau of Soils, Dept. Agriculture.

[4] Bulletins 155 and 160, Colorado Agricultural Experiment Station.

> "It would seem that di-hydroxystearic acid is either a direct or an indirect factor in the low productivity of soils."

Several other organic compounds have since been separated from the soil by him, which in liquid cultures exert a pronounced retarding influence upon wheat seedlings grown therein.

The source of these compounds has been variously attributed. Reference has already been made to the root excretions as the responsible agent for their origin. Beyond the evolution of CO_2 however, no evidence has been secured to indicate such excretions from plant roots. Czapek, for example, was unable even by the most sensitive and delicate methods, to demonstrate the presence of any organic acids in plant-root excretions. That these organic substances are formed as a result of the decomposition of the plant residues in the soil, due to the agency of soil bacteria, is an explanation of the problem that is relatively new but one which, because of its plausibility, is rapidly gaining support. Among the first to suggest this possibility was Dachnowski[6] in connection with his work on the growth of seedlings in peptone solutions inoculated with various soil organisms.

It is reasonable to assume that continuous cropping will deposit large quantities of plant tissue of a specific character in the soil. Further, that these tissues will undergo decomposition with the formation of by-products characteristic for the crop grown. Decomposition of wheat refuse undoubtedly will yield different by-products than clover or corn-stubble. Thus the continued one-cropping would tend to cause an accumulation of by-products which may prove toxic to the particular crop grown, whereas they may not affect other crops. By changing the rotation these would be destroyed so that when the original crop is again grown no toxicity would exist. Another possibility which presents itself is the fact that a specific type of by-products may tend to favor certain species of bacteria, which would cause a disturbance in the equilibrium of the soil flora. Brown[7] has very recently attempted to determine the influence of various crop rotation systems on the nature of the soil bacterial flora. He has shown that a two- or four-year rotation tends to maintain a higher ammonifying and nitrifying coefficient on the part of the soil than continuous one-cropping.

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[5] Schreiner, Bulletin 80, Bureau of Soils, U. S. Dept. Agriculture.

[6] Dachnowski, Contributions 53, Botanical Laboratory, Ohio State University.

Another aspect to this problem of the reduction in crop yields due to continuous cropping is presented by the statements of Bolley,[8] in which he endeavors to explain the cause of a type of "soil sickness" prevalent in the north-central states of the Mississippi valley. Here repeated one-cropping has been largely practiced with wheat and flax respectively. The result has been a very marked decrease in the crop yields. This decrease seems hardly explicable on the basis of a rapid reduction in the supply of available plant food, although this is the explanation which has invariably been given for this phenomenon. Reference has already been made to de Candolle's explanation, namely that the reduction in crop yield is not due to a starvation, but rather to a poisoning occasioned by the accumulation of plant-root excretions. Bolley, however, claims that constant one-cropping permits the possibility of an accumulation, not of toxins, but rather of parasitic fungi and spores pathogenic to the crop constantly grown. On this basis, after repeated one-cropping, the soil becomes more or less extensively contaminated with such disease-producing fungi. Such a contamination with disease-producing factors would mean an increased percentage of sick and infected plants with a resulting diminution in crop yield. It is evident that Bolley does not attribute the reduced yield to a decrease in available plant food, and in this respect his explanation harmonizes with the view held by Cameron and Whitney,[9] who are responsible for the following statement:

"Soils are unproductive not because of the lack of the proper nutrients, but because they contain substances actually deleterious to plant growth."

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[7] Brown, Tech. Bulletin No. 6, pt. II, Iowa Agricultural Experiment Station.

[8] Bolley, Science, Oct. 21, 1910.

[9] Cameron and Whitney, Bulletins 22 and 40, Bureau of Soils.

Bolley as it were, explains the matter on the basis of an accumulation of disease organisms; Cameron and Whitney on the accumulation of deleterious waste products.

But to return to the work of the Bureau of Soils, more particularly the work of Schreiner. It is further claimed that the above deleterious substances are toxic and are the result of root excretions. Statements are thus made that "continuous cropping tends towards an accumulation of the waste products of plants, resulting in reduced crop yields"; that "these waste products are evidently of a soluble nature for when soil extracts are filtered through a Berkefeld filter, the toxic, retardative action of the soil persists in the filtrates"; evidence which would seem to indicate that the reduced yields are not directly due to the bacterial flora but rather to the chemical nature of the soil solution. The addition of lampblack and occasionally of $CaCO_3$ removes this toxic property, in which case seedlings grown in extracts so treated apparently make good growth. This evidence is indicative at least, that the retardative action is not due to a lack of plant food but rather to the presence of some chemical substance of a toxic nature which is removed from the sphere of action by the lampblack, and in some cases even by $CaCO_3$.

Considerable adverse criticism, notably that of King[10] has been directed against the methods employed by the Bureau of Soils for the attainment of these results. King claims that the quantities of the various toxic substances added to the soil filtrates in the Bureau's experiments are of such proportions that even the smallest amount used would be equivalent to 70 pounds per acre foot. If present in such quantities, he argues it should be an easy matter to isolate these substances from the soil, whereas, as a matter of fact, it is necessary to resort to the most elaborate methods to find even traces of these substances in the soils.

At this point reference should again be made to Bolley's hypothesis. If his explanation is correct, then filtration through a Berkefeld should remove the disease germs or their spores and thus render the filtrates innocuous so that they should support plant growth as efficiently as a sterilized filtrate from the same soil. As far as the author is aware this has not been tried out, but deserves further consideration before final acceptance of Bolley's hypothesis can be warranted.

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[10] King—Science, vol. 27, 1908, p. 694.

Greig-Smith[11] claims to have shown that volatile antiseptics are beneficial to such toxic soils. He claims that the former dissolve out and absorb the toxic ingredients and remove them from the sphere of activity, the so-called rhizoplane or root zone. He thus accounts for the beneficial action commonly secured when soil is treated with CS_2. Heating of soil as practiced in the partial sterilization of soil is likewise supposed to eliminate the toxic action or rather detrimental action manifested by such soils. Russell and Hutchinson[12] attribute the beneficial action of partial sterilization or antiseptification of soil to the destruction of certain forms of life which ordinarily prey upon the bacteria concerned in the conversion of unavailable plant food to available forms. They regard the protozoa, amœba and ciliates as competitive factors in the soil. If removed, the bacteria have full control and so can continue their multiplication with subsequent elaboration of plant food.

It is thus apparent from the above discussion that a multiplicity of hypotheses have been advanced to explain various phenomena manifested by soils which appear to give reduced crop yields. No doubt one can not attribute to any single factor the responsibility for the reduced productivity. Instead it appears far more probable that many factors contribute and coöperate to bring about aforesaid phenomena. In the light of the very complex nature of the soils, this seems the more probable and the only reasonable view to take relative to the problem in hand.

On the basis of the numerous explanations regarding this matter or closely related phenomena advanced by various investigators, it would appear that the soil bacterial flora must be regarded as a most important agent, responsible, at least in part, directly or indirectly, for the reduced crop yields on continuously cropped soils. Their action must undoubtedly center in their ability to decompose the various vegetable debris constantly being returned to the soil.

That the bacterial flora of the soil may be influenced by continuous one-cropping and that the bacteria in turn may influence subsequent crops, either the same or different, by the production of by-products harmful or beneficial to the plants, are matters which have as far as is known been largely ignored in considerations of soil toxicity problems. Very recently, however, Kellermann has called attention to the possible interrelationship existing between the soil bacterial flora and the growing crop.[13] It seems plausible to expect such an interreaction between the crops and the soil bacteria. We know how very sensitive bacteria are to slight variations in the composition of culture media. Whether in the cosmopolitan life of the soil the variations occurring in the composition of the soil due to the accumulations of crop residues may affect that life, has not been demonstrated, but is none the less most probable. Lyon and Bizzell[14] have suggested the influence of certain crops on nitrification which would thus influence subsequent crops.

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[11] Centbl. Bakt., 2 Abt., vol. 30, pp. 154, 156.

[12] Russell and Hutchinson, Journal Agricultural Science, 1911, vol. 3, p. 111; 1912, vol. 5, pp. 27, 86; 1913, vol. 5, p. 152.

Examination of soils in close proximity to the root systems of various plants reveals a marked difference in the quality as well as in the quantity of the bacteria in contrast to that found at some distance from the roots. (See Table I.) There is invariably a much higher germ content in the immediate vicinity of the roots. Thus of 32 determinations made on soils adjacent to and remote from the roots of various plants, all but five showed a much higher germ content near the roots than at some distance away. Totalling the 27 counts with an increase near the roots, one finds almost three times as many bacteria near the roots as at some distance therefrom. One must concede accordingly that the root system of plants exert a marked effect upon the bacterial flora of the soil in the immediate vicinity of the roots. Whether this influence is due to the respiratory products of the roots or to the products formed as a result of the decomposition of the root cells which are more or less constantly being sloughed off, the author is unable to say. Stoklasa has likewise shown that on the same soil under different crops the germ content shows marked variations. Thus:

Sugar beet soil	3-5 million.
Barley soil	1-2 million.
Clover soil	7-8 million.

The work herein reported endeavors to throw some light on this subject of reduced crop yields and soil toxicity. The author maintains that when a particular plant species is continually grown, the plant residues left in the soil occasion a change in the chemical composition of the soil, which in turn influences the bacterial flora. Certain plant species will favor certain types of bacteria and inhibit others and thus disturb the bacterial equilibrium of the soil. The new flora thus established then produces specific changes in the composition of the soil which affect subsequent plant growth, favoring some plant species and retarding others.

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[13] Kellermann, Cir. 113, Bureau of Plant Industry, U. S. Dept. Agriculture.

[14] Jour. Indust. and Engin. Chem., 5, 1913, No. 2, p. 136.

The problem as stated above is complex and presents many difficulties. With the three variable factors, crop, soil and bacteria, it was realized that little more than substantiation of the principles above set forth, in a few specific instances could possibly be hoped for. The establishment of the fact of such an interrelationship between soil crop and soil bacteria, was deemed worthy of an attempt.

On the basis of Schreiner's work, it was assumed that extracts made from sterilized soils in which different types of bacteria had been grown, would show differences when employed as nutrient solutions for the growth of various types of plant seedlings.

"The good or bad properties of a soil as shown by the growth of plants upon it, are largely transmitted to the aqueous extract of that soil."[15]

In the same manner a soil continuously cropped with one plant must show differences when employed in extract form for the growth of soil organisms.

"We must regard the excreta of the growing plant roots as one of the main causes of the low yields obtained in improper rotation of crops. By alternating the crops one invariably secures larger yields than where one crops continuously with a given crop."[16]

These two principles were employed as the basis for the work herein reported: First, to ascertain in individual cases what influence the growth of different pure cultures of soil organisms in sterile soil will have upon certain plant seedlings grown in the extracts of such soils; second, to ascertain what influence the growth of different plants in the same soil may have upon pure cultures of bacteria grown in the extracts of such cropped soils.

Considerable difficulty was experienced in the use of the ordinary perforated cork for the growth of the seedlings. After some experimentation, the paraffin block method [17] was devised and subsequently used in all work herein reported.

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[15] Bureau of Soils, Bulletin 28, p. 21.

[16] Bureau of Soils, Bulletin 40, p. 40.

Several preliminary experiments were performed in an endeavor to ascertain whether bacterial growth would modify the soluble chemical content of the soil solution as determined by the effect produced upon the growth of seedlings in the respective soil extracts. The general procedure employed was as follows:

For any given soil, 1000-gram portions of air-dry soil in large glass containers, were sterilized in the autoclave. In all cases one 1000-gram portion was left unsterilized to secure a control on the action of the normal soil flora. After cooling, the respective containers were inoculated with equal quantities of suspensions of the organisms employed, and, in addition, one with a suspension of the normal soil. The unsterilized soil sample received the same quantity of liquid used above, but in a sterile condition. Sufficient water was always added to give a moderately moist condition. The per cent employed was, of course, dependent upon the nature of the soil, relatively small quantities being employed for the sandy soils whereas the marsh and peat soils received a comparatively large per cent.

In addition to the above a sterile control for each soil was employed. This was made by adding to one container of sterilized soil, the same quantity of sterilized water which was employed in the preparation of the inoculating suspensions for the other containers.

The soil cultures thus prepared were incubated at room temperature for not less than seven days and usually for two to four weeks. To the cultures 1000 cc. of distilled water were then added. In the earlier work the mass was thoroughly and vigorously shaken and then filtered through a clean Berkefeld filter. In filtering it was found more expedient to add the soil-water mixture directly, rather than to attempt to siphon off the more or less clear supernatant liquid which collected on standing, and then filtering. Filtration was far more rapid. The soil added in the first method appeared to aid in the filtration and to retard the clogging-up of the Berkefeld. In the later work, however, it was found just as efficient to filter through ordinary filter paper, using Schleicher and Schüll's

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[17] C. Hoffmann, Botanical Gazette 55, No. 3, Mar. 1913. Centbl. Bact., 2 Abt., Bd. 34, 1912.

No. 588 folded filter for the purpose. The residue soils on the filter were washed with sufficient distilled water to make the filtrates up to 1000 cc. These were invariably clear, golden straw in color, and to all appearances would invariably be mistaken for ordinary bouillon. The extracts were then sterilized.

Five hundred cc. portions were then placed in pint Mason jars (a hydrometer cylinder being, however, a far more satisfactory container). In these were placed the paraffin blocks containing the plant seedlings. (Plate XXIII.) (The latter were secured in essentially the same manner as that employed by the Bureau of Soils.) The water cultures thus prepared were placed in the greenhouse to permit the growth of the plants. The water lost by evaporation and transpiration was restored in the form of sterile, distilled water.

All cultures were thus identical in every respect with the exception that different organisms had grown in the samples of soil from which the extracts had been made. Thus in the first series a field soil was employed. Of this 1000-gram portions were sterilized and then inoculated according to the following scheme:

1. Yellow ammonifier.
2. Ps. radicicola (garden pea).
3. Azotobacter.
4. B. prodigiosus.
5. B. liquefaciens fluorescens.
6. Cladothrix odorifera.
7. Aspergillus niger.
8. Sterilized soil.
9. Normal soil.
10. B. subtilis.

After 14 days' incubation, extracts of the respective soils were made according to method outlined above. These were then employed as nutrient solutions for the growth of wheat seedlings. The results are perhaps best apparent from Plate XXIV, which shows the condition of the seedlings after 18 days of growth. One observes a wide difference particularly in the root development of the seedlings in the various extracts. A most extensive root development occurred in the case of the extracts from the ammonifier, azotobacter and B. liquefaciens fluorescens. 1-3-5. (Plate XXIV.) In contrast to these, the seedlings in the normal soil extract, and B. subtilis show a very sparse root development. It is suprising, however, that despite these pronounced differences in root development, there should be such slight differences in the developments of the tops.

In another test corn seedlings were employed with extracts from samples of black marsh soil in which the following organisms were grown:

1. Azotobacter.
2. B. denitrificans.
3. Proteus vulgaris.
4. Normal soil.
5. Cladothrix odorifera.
6. Yellow ammonifier.
7. Manure organism (B. mycoides?)
8. Sterile soil.

In this series Cladothrix odorifera and Proteus vulgaris occasioned a more luxuriant development in the case of corn and a marsh soil. In the case of the denitrifier the poorest development of the corn seedlings was secured. Here again the differential influence seemed to be confined more to the root than to the leaf system.

In this manner a large series of experiments (20) have been performed in which one or the other of the three factors involved has been modified. As typical of the results secured in these experiments, the data in tables II, III and IV are presented. One finds here marked variations both in the size of crop and in the dry weight of same as influenced by the various cultures. It is interesting to note that the manured soils (5 per cent finely ground dry manure) invariably show a larger gram yield than the unmanured soils; the two exceptions being in the case of corn and clover in the extracts made from the soil inoculated with Proteus vulgaris. If one measures the influence of the organisms by the weight of crop grown upon the extracts, one finds marked variations. In duplicate experiments where all three factors were identical, similar results are invariably secured, showing that whatever influence is exerted, the same is constant as long as the conditions remain the same. A change in any one of three factors occasioned a change in the influence exerted. Note the much larger yield of corn on the manured soil with B. prodigiosus in contrast to that with Proteus vulgaris. The yield of clover with the yellow ammonifier on the manured soil is four times as great as that in the sterile manured soil. These are variations which are well beyond the factor of error. It is apparent that individual organisms when permitted to grow in pure culture in soils, influence the composition of the soil solution sufficiently to reveal an effect upon the crops grown in such solutions. On the supposition that continued one-cropping will modify the bacterial flora of the soil by favoring some forms and hindering others, it seems reasonable to expect that such a change of the flora will exert an influence upon subsequent crops.

THE EFFECT OF CROPS ON THE GROWTH OF ORGANISMS IN SOIL EXTRACTS.

Experiments were next performed in an endeavor to show that certain crops can so modify the soils' composition as to favor certain types of organisms and perhaps retard others. These were carried on in the following manner:

Three distinct types of soils, sand, loam and marsh, were employed. For each soil eight pots were filed with 3000 grams of soil. These were then planted to corn, oats and clover, using two pots to each soil for each crop; the fourth set being left unsown to serve as controls on the respective soils.

After luxuriant growth had occurred with penetration of the root systems throughout the soil mass (about six weeks' growth), the soil was shaken free from the roots, run through a ¼-inch sieve and then air-dried at 37° C. Of the dry soil so obtained, 1000-gram portions were vigorously shaken with 1000 cc. of distilled water and then immediately filtered through paper as previously described. In this manner between 600 and 800 cc. of clear extract were secured from each cropped soil. In the case of the marsh soil 1200 cc. of water were employed, owing to its large water-holding capacity.

The extracts thus secured were then measured with a burette into test-tubes in 10 cc. quantities. After plugging, these tubes were sterilized and subsequently used as culture media for the growth of pure cultures of soil bacteria. The rate of multiplication of the organisms in these extracts was used as an index of the influence, whether beneficial or detrimental, which the respective extracts exerted.

This was accomplished by making a suspension of a young culture of the specific organism under consideration in 100 cc. of sterile distilled water. After vigorous shaking, the faintly turbid liquid was filtered aseptically through glass wool, to insure a uniform and homogeneous suspension of the organisms. The 10 cc. quantities of the respective sterile soil extracts were then inoculated with these suspensions at the rate of 0.1 cc. per tube. For each extract six tubes were thus inoculated. By making germ content determinations on one tube a day, some idea as to the rate of multiplication of the organism in the respective soil extracts, could be secured. These determinations were made daily for a period of five days by which time most of the organisms employed had reached their maximum numbers. Plain gelatin was used as plating medium in some of the experiments, plain agar in others. The dilutions were so adjusted as to give plates upon which the colonies could be readily counted. In all cases an actual count of all the colonies on the plates was made, rather than estimating the total number by counting only a section of each plate.

In the tables which follow, Tables V to VII, the data secured in a few of these experiments are presented. These have been further arranged graphically in Plates XXV-XXVII which show more clearly the influence exerted by the various crops on the soil extracts. It will be noted that on the whole the results are consistent. This is very strikingly the case with the marsh soil (Plate XXV), where with all organisms employed the rate of multiplication was greatest in the corn-cropped soil, the other soils ranking as follows: clover-cropped, oat-cropped and control. This same order prevails with the three organisms here examined. Apparently the growing of the crops upon the marsh soil changed the composition of the soil solution in such a way as to enhance the growth of the bacteria in the extracts of the same.

With the loam soil, the growing of crops seems to have modified the soil solution so as to cause a decreased multiplication of the bacteria in the extracts in contrast to the control, which showed the most pronounced growth. With sand there is a very marked retardative influence evident, due to the cropping of the same with corn and oats. This applies to all three organisms. The normal in this case as with the loam, shows the most marked development with the exception of the ammonifier, where the clover-cropped soil exceeds the normal. In all three cases, the normal and the clover-cropped soils show far more multiplication of the bacteria in their respective extracts than the corn or oat-cropped soils. On the basis of these data it appears as if the soil is the more important factor. The marsh soil when cropped enables a greater multiplicaton of the bacteria than the control or non-cropped soil. The reverse seems to be true with the loam and the sand soils here employed. The three organisms here experimented with are all similarly affected. Azotobacter, B. denitrificans, Sarcina lutea, B. liquefaciens fluorescens have also been used, but owing to contamination of the plates, the results have been ignored. It was possible, however, to note that on these plates the same relationship existed as above; invariably the corn-cropped marsh soil showed the most pronounced multiplication. In fact all the marsh soils which had been cropped showed in their respective extracts this stimulating effect on the growth of these organisms. In sand and loam, however, cropping appeared to exert a retarding influence on the growth of bacteria in the extracts from these soils.

In tables VIII to X further similar data are summarized which show the same general characteristics described above. They give added substantiation to the hypothesis previously stated, that growing crops modify the soil solution so that the latter exerts a more or less pronounced influence, stimulative or retardative, upon the bacterial species of the soil.

SUMMARY.

The data which have been secured in the experiments described above, enable one to make the following conclusions:

1. The growth of individual species of bacteria in a soil produces changes in the soil solution which manifest themselves by an increased or decreased development of plant seedlings when grown in extracts made from such soils.

Invariably this influence seems to affect the root development rather than the leaf development.

In repetitions of the same experiment consistent results are secured as long as all three factors, soil, bacterial species and crop, are the same. A change of any one factor modifies the results secured.

2. The growth of individual species of crops in a soil produces changes in the soil solution which manifest themselves by an increased or decreased bacterial multiplication in extracts made from such soils.

The extracts made from a marsh soil cropped by corn, oats or clover in all cases stimulated bacterial multiplication. This stimulation was consistently greatest in the case of the corn-cropped soil. In contrast to the marsh, the extracts from the cropped loam and sand soils invariably retarded the multiplication of the bacteria grown in the same.

3. There is a definite relation between the growth of crops in soil and the growth of bacteria therein. It is possible that one crop in a given soil may stimulate certain bacterial species which in turn will produce changes in the composition of the soil solutions. These changes may or may not influence beneficially subsequent crops.

Owing to the variability of the three factors involved, soil, crop and bacterial flora, it is impossible to establish any hard and fast laws for all cases.

It seems evident from the work that if these relationships could be established, one would have a definite basis on which to determine crop rotation systems. If corn favors the development of organisms whose growth stimulates oats, then oats can follow corn in a rotation; whereas, if corn retards the development of species favorable to oats or favors others detrimental to oats, then corn should not precede oats in a crop rotation.

Future work along the lines suggested by this paper should throw much light on this all-important subject.

TABLE I.—Showing influence of proximity to plant roots upon germ content of the soil.* Bacteria per gram.

Plant.	Adjacent to roots.	†Remote from roots one or more feet.
Currant	1,445,000	1,270,000 +
Asparagus	2,200,000	675,000 +
Grass	29,850,000	5,450,000 +
Iris	5,150,000	4,800,000 +
Raspberry	4,560,000	1,890,000 +
Rhubarb	3,600,000	970,000 +
Cabbage	8,085,000	740,000 +
Plum	1,335,000	595,000 +
Apple	3,200,000	1,345,000 +
Pea	1,770,000	1,520,000 +
Tomato	790,000	525,000 +
Rhubarb	1,035,000	465,000 +
Bean	63,500	59,500 +
Tomato	1,086,000	530,000 +
Sunflower	164,000	1,105,000 —
Potato	385,000	410,000 —
Potato	500,000	670,000 —
Cabbage	245,000	325,000 —
Cauliflower	213,000	504,000 —
Rhubarb	270,000	80,000 +
Tomato	565,000	80,000 +
Asparagus	84,000	66,500 +
Currant	275,000	177,500 +
Gooseberry	108,000	80,500 +
Hemp	6,500	500 +
Squash	930,000	114,000 +
Mangel	31,000	16,000 +
Tobacco	61,500	6,500 +
Strawberry	40,000	28,000 +
Cabbage	62,500	9,000 +
Grape	131,000	120,000 +
Cauliflower	72,000	40,500 +
Total	67,313,000	24,667,500

TABLE II.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of corn seedlings.

ORGANISM.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight. grams.
Normal soil flora	22.5	25.0	47.5	1.665	17.5	25.0	42.5	1.652
Sterile soil	28.7	25.0	53.7	1.788	20.0	27.5	47.5	2.025
Cladothrix odorifera	32.5	27.2	54.7	1.356	12.5	25.0	37.5	1.610
Azotobacter	30.0	20.0	50.0	1.590	17.5	27.5	45.0	2.188
B. prodigiosus	20.0	20.0	40.0	1.560	23.7	25.0	48.7	2.237
B. proteus vulgaris	18.7	22.5	41.2	1.833	8.7	22.5	31.2	1.135
Ammonifier	20.0	21.7	41.7	1.304	17.5	25.0	42.5	1.946
Normal soil	20.0	20.0	40.0	1.264	15.0	30.0	45.0	2.153
B. denitrificans	32.5	21.2	53.7	1.311	20.0	27.5	47.5	2.105

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* These results are compiled from data secured by Mr. B. Hammer and Mr. Eldredge.

† Greater number adjacent to roots indicated by + ; less number by —.

TABLE III.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of wheat seedlings.

ORGANISM.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.
Normal soil flora	30.0	12.5	32.5	0.698	28.7	17.5	46.2	1.128
Sterile soil	22.5	13.7	36.2	0.750	27.5	17.5	45.0	1.060
Cladothrix odorifera	26.2	16.2	42.4	0.779	21.2	20.0	42.2	1.295
Azotobacter	27.5	15.5	43.0	0.700	23.7	17.5	41.2	1.151
B. prodigiosus	27.5	17.5	45.0	0.841	27.5	18.7	46.2	1.358
B. proteus vulgaris	25.0	15.0	40.0	1.038	27.5	18.7	46.2	1.171
Ammonifier	24.2	15.2	39.4	0.952	23.7	20.0	43.7	1.574
Normal soil	30.0	18.7	48.7	1.142	25.0	21.2	46.2	1.250
B. denitrificans	23.7	15.5	39.2	0.689	22.5	16.2	38.7	0.901

TABLE IV.—Influence of soil extracts from sterilized soils in which certain organisms had been grown upon the growth of clover seedlings.

ORGANISM.	Normal soil.				Soil + 5% manure.			
	Root length, cm.	Leaf length, cm.	Total length, cm.	Dry weight, grams.	Root length, cm.	Leaf length, cm	Total length, cm.	Dry weight, grams.
Normal soil flora	10.0	1.2	11.2	0.109	13.7	4.3	18.0	0.190
Sterile soil	15.0	3.7	18.7	0.222	6.8	4.1	10.9	0.103
Cladothrix odorifera	16.2	3.1	19.3	0.320	12.5	6.2	18.7	0.446
Azotobacter	15.0	3.7	18.7	0.202	3.7	3.7	7.4	0.153
B. prodigiosus	16.2	4.3	20.5	0.188	8.7	3.7	12.4	0.340
B. proteus vulgaris	15.0	4.3	19.3	0.215	4.3	2.5	6.8	0.182
Ammonifier	15.0	4.3	19.3	0.110	12.5	6.8	17.3	0.543
Normal soil	7.5	1.2	8.7	0.194	12.5	6.8	19.3	0.434
B. denitrificans	12.5	4.3	16.8	0.179	8.7	5.6	14.3	0.441

TABLE V.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the ammonifying organisms. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh	Control	169,000	128,600	1,370,000	1,800,000	2,005,000
	Corn	167,500	455,000	4,000,000	66,400,000	25,000,000
	Oats	131,000	259,300	4,300,000	4,800,000	6,500,000
	Clover	127,500	2,060,000	7,000,000	16,100,000	11,400,000
Loam	Control	123,000	460,000	4,500,000	40,400,000	10,400,000
	Corn	139,000	2,175,000	5,700,000	11,900,000	11,900,000
	Oats	160,000	3,205,000	11,300,000	16,600,000	10,200,000
	Clover	137,500	810,000	4,400,000	6,200,000	5,300,000
Sand	Control	189,500	6,900,000	17,200,000	16,200,000	23,800,000
	Corn	118,500	112,000	570,000	325,000	5,600,000
	Oats	132,500	45,000	2,295,000	5,800,000	9,900,000
	Clover	175,000	7,300,000	31,400,000	31,400,000	21,200,000

TABLE VI.—Influence of the extracts of variously cropped soils upon the rate of multiplication of B. prodigiosus. Number of bacteria per cc. of extract.

Soil.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh	Control....	238,500	59,000	234,000	420,000	4,900,000
	Corn.......	350,000	910,000	43,600,000	82,400,000	22,600,000
	Oats.......	286,500	57,000	387,000	2,210,000	11,500,000
	Clover.....	470,000	86,000	15,500,000	23,000,000	34,800,000
Loam	Control....	231,500	900,000	21,700,000	19,700,000	17,400,000
	Corn.......	201,000	84,000	1,770,000	20,300,000	14,300,000
	Oats.......	302,000	80,300	1,380,000	8,700,000	13,300,000
	Clover.....	110,000	81,000	490,000	4,400,000	13,400,000
Sand	Control....	287,000	5,200,000	45,300,000	38,200,000	16,000,000
	Corn.......	241,000	291,600	2,910,000	7,500,000	11,300,000
	Oats.......	510,000	22,000	268,000	6,700,000	8,600,000
	Clover.....	284,000	435,000	25,100,000	26,600,000	15,700,000

TABLE VII.—Influence of the extracts of variously cropped soils upon the rate of multiplication of B. proteus vulgaris. Number of bacteria per cc. of extract.

Soil.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.
Marsh......	Control....	119,000	1,515,000	7,100,000	10,500,000	5,300,000
	Corn	107,500	9,600,000	13,900,000	43,200,000	43,300,000
	Oats.......	114,500	16,500,000	9,200,000	10,200,000	11,700,000
	Clover.....	109,000	17,500,000	24,700,000	26,900,000	18,800,000
Loam ..	Control	103,000	10,400,000	16,000,000	13,300,000	23,400,000
	Corn.......	118,000	9,000,000	15,600,000	14,200,000	12,700,000
	Oats.	99,000	8,500,000	19,700,000	19,500,000	10,600,000
	Clover.....	108,000	1,250,000	10,100,000	8,800,000	8,100,000
Sand..................	Control....	78,000	19,800,000	37,800,000	24,700,000	19,800,000
	Corn	80,000	7,500,000	7,100,000	10,000,000	8,800,000
	Oats.......	87,000	3,370,000	7,700,000	10,100,000	7,400,000
	Clover.....	95,000	13,200,000	14,700,000	16,400,000	11,300,000

TABLE VIII.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the ammonifying organisms. Number of bacteria per cc. of extract.

Soil.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh....	Control....	25,600	9,800,000	16,700,000	3,800,000	910,000	5,100,000
	Corn ..	81,000	9,000,000	97,000,000	137,000,000	14,400,000	65,000,000
	Oats.......	15,950	1,435,000	99,000,000	97,000,000	47,800,000	72,900,000
	Clover.....	40,900	930,000	61,000,000	115,000,000	106,000,000	57,000,000
Sand	Control....	50,600	2,775,000	11,400,000	110,000,000	3,900,000	3,400,000
	Corn.......	124,500	3,340,000	14,400,000	....	13,500,000	6,400,000
	Oats. ..	95,000	2,170,000	7,800,000	4,040,000	150,000	260,000
	Clover ...	147,500	1,370,000	7,800,000	24,200,000	1,060,000	8,900,000
Loam....	Control .	100,000	3,560,000	22,500,000	6,300,000	8,000,000	21,300,000
	Corn .	80,600	790,000	28,800,000	39,000,000	49,000,000	54,000,000
	Oats.......	26,400	1,315,000	20,600,000	16,400,000	8,400,000	25,700,000
	Clover....	67,000	6,200,000	19,700,000	10,200,000	20,900,000	16,700,000

TABLE IX.—Influence of the extracts of variously cropped soils upon the rate of multiplication of one of the denitrifying organisms. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh	Control....	21,900	131,000	24,900,000	15,300,000	490,000	70,000,000
	Corn.	18,300	53,000	50,400,000	101,600,000	41,200,000	70,000,000
	Oats.......	8,050	139,000	49,500,000	57,000,000	2,620,000	100,000,000
	Clover	11,550	226,000	32,000,000	61,700,000	24,100,000	70,000,000
Sand	Control	13,200	197,000	44,800,000	36,900,000	90,000	35,500,000
	Corn.......	11,600	89,000	21,600,000	18,800,000	21,200,000	49,200,000
	Oats.......	16,700		27,100,000	15,500,000	2,480,000	27,700,000
	Clover	17,700	101,000	39,900,000	29,600,000	23,600,000	27,200,000
Loam	Control....	9,500	70,000	32,800,000	39,400,000	59,200,000	37,900,000
	Corn.......	9,100	29,000	49,000,000	49,800,000	55,800,000	38,900,000
	Oats	9,000	162,000	41,400,000	50,100,000	13,600,000	13,100,000
	Clover	9,800	1,500	28,800,000	23,700,000	30,300,000	15,200,000

TABLE X.—Influence of the extracts of variously cropped soils upon the rate of multiplication of Sarcina lutea. Number of bacteria per cc. of extract.

SOIL.	Crop grown.	Initial.	First day.	Second day.	Third day.	Fourth day.	Fifth day.
Marsh....	Control..	500	800	300	700	200	300
	Corn	300	300	10,600	42,000	90,000	148,000
	Oats	300	700	3,400	63,000	67,000	84,000
	Clover	400	600	1,300	24,900	68,000	93,000
Sand	Control	500	200	7,100	16,700	500	28,100
	Corn	500	700	400	4,700	25,800	
	Oats.......	700	500	700	3,300	1,800	11,400
	Clover.....	500	300	700	7,200	11,700	20,300
Loam....	Control	200	700	400	20,700		32,400
	Corn ..	500	500	2,900	12,600	44,000	39,200
	Oats.......	400	400	300	16,100	15,600	35,300
	Clover.....	500	100	1,300	4,600	3,000	4,500

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CONTENTS:

Some Methods of Embryological Technique, by....*Bennet M. Allen.*

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Some Methods of Embryological Technique.

BENNET M. ALLEN.

Contribution from the Zoölogical Laboratory No. 210.

THERE are two distinct lines of work to be outlined in this paper. These are of especial value not only in research work, but in the preparation of material for laboratory instruction.

The writer has long felt that there should be some satisfactory method of preserving fine dissections, free-hand sections, opaque eggs, etc., in such a manner that they could be studied by reflected light under the low power of the microscope. It has become the almost universal practice to clear an object and to mount in some such substance as Canada balsam, glycerine jelly, etc. The study of small opaque objects has been too greatly neglected.

Experiments were made in imbedding entire frog eggs. At first glycerine jelly was used, but this was found to be altogether unsatisfactory owing to its clearing properties. This is precisely the thing that we wish to avoid. So gelatine alone was employed with very satisfactory results. This is made up as follows: First of all thymol was dissolved in distilled water to saturation. In accomplishing this heat is employed. If the solution becomes murky upon cooling, it is filtered until clear. The gelatine should be prepared in the usual way—that is, it should be allowed to absorb as much as possible of this thymol water, and the excess water is drained off. Then it is ready to be heated and used.

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The gelatine solution is kept in test tubes that can be corked, and when desired for use these are placed in a water bath. The liquefied gelatine is then poured out on the slide and allowed to harden for a few minutes. In order to make cells suitable for mounting large objects, strips of glass are pressed upon a slide in a manner indicated in the diagram. The liquefied gelatine is poured into the space in the center. Within a few minutes after solidification, a hot needle is used to melt small points in the gelatine into which the objects to be mounted are then inserted. In this manner they can be placed in any arrangement desired, and held in position with the needle until the surrounding gelatine solidifies.

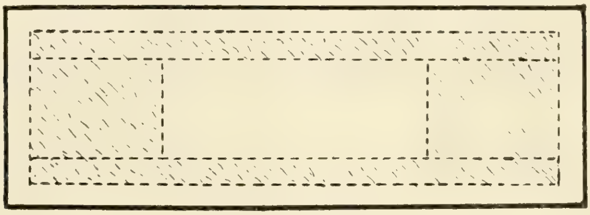
Fig. 1

It is thus possible to mount as many as fifteen or twenty properly arranged frog eggs in different stages of segmentation. Care should be exercised to have the gelatine deep enough in the cell to almost fill it up. After allowing the gelatine to cool to such a degree that all of the objects are held firmly in place, gelatine heated barely enough to have a liquid consistency, is then poured into the cell in sufficient quantity to flood it, and a slightly warmed slide is placed upon the cell in such a manner as to coincide in position with the slide below. In preparing it, care must be exercised to prevent the inclusion of air bubbles in the cell, and at the same time furnish a complete film of melted gelatine between the cover slide and the underlying glass strips. This slide should be held in place until the gelatine has solidified, thus holding it firmly in position.

It is next necessary to seal the mount. This is done by first cleaning out the excess gelatine from the grooves formed by the strips between the margins of the two slides and the edges of the strips between them. This can be done with a small piece of wood like a toothpick. Care should be taken to clean away the gelatine quite thoroughly. It is even advisable to cleanse the groove with a moist cloth or camel's hair brush. It should then be thoroughly dried in like manner. All of this requires care and patience, and the permanency of the mount depends upon the neatness with which it is done.

Then some cement, such as gold size or asphaltum, is run into the groove along all four sides, and the preparation is set aside for the cement to harden. From day to day additional cement is added, until by its successful solidification the groove is completely filled up. The preparation should then be permanent, with the precaution that it should not be subjected to heat such as might be caused by placing it where direct sunlight will fall upon it. The gelatine can be hardened by the simple process of previously infiltrating the objects to be mounted with a solution of formalin. After mounting, this will gradually diffuse out into the gelatine and harden it.

This method can be of quite wide application. It could be used in free-hand sections, for instance, one can cut in half the blastulæ and gastrulæ of the frog. It has always been a problem to properly preserve the halves of such gastrulæ that one obtains by cutting the egg in two with a safety-razor blade. Preparations showing various stages of gastrulation were mounted in the manner described, and proved very instructive. Embryological or other objects might well be cut into thick opaque free-hand sections. These should prove extremely instructive, opening up new and very important fields of embryological work.

Various modifications of the foregoing method may be used. It is quite possible to use hollow-ground slides designed for use with a hanging drop. In such cases as flooding the cell at the completion of mounting, one can use a circular cover slip to cover it, and this can be ringed on a turntable in the usual manner. One must exercise great precaution to avoid overheating the gelatine. When a needle is used to heat a place for the insertion of one of these objects, one should give a little time to allow the gelatine to almost solidify, the object can then be placed in position and blown upon, or the slide placed upon ice so that solidification may be greatly hastened. In this way it is often possible to imbed rather delicate objects with very large cavities, such as the medullary fold stages of Ambystoma.

In making preparations of this kind, where deeply pigmented specimens are used, it is often found desirable to bleach them. Beautiful preparations of the segmentation stages of the frog can be made by the methods explained above in which the material has been prepared by immersion in hydrogen peroxide—the ordinary commercial strength can be used. It is only applicable with specimens that have been thoroughly hardened in any of the ordinary hardening fluids, or even in formalin. In the latter case care must be taken with delicate structures to wash out the formalin before putting them in the hydrogen peroxide, otherwise the tissues would be distorted by the oxygen liberated. Frog eggs may be left in the hydrogen peroxide for even two or three weeks, if necessary. My experience has been that about a week will suffice for the bleaching of the pigmented area to a light brown color.

When this has been accomplished to the right stage, the segmentation furrows show with remarkable clearness. One great difficulty in using the frog as a type form for a study of elementary embryology has been the fact that it has appeared impossible to make whole mounts of the young tadpoles. Well-fixed specimens in all stages of development can be completely bleached with hydrogen peroxide and rendered as white as chick embryos. They can then be stained and mounted in balsam. My experience has been that a very dilute stain of alum cochineal gives the best results, although I have also used a dilute stain of Conklin's hæmatoxylin. Emphasis must be laid upon the dilution of these stains. In the case of both, they are diluted with distilled water until they show but a faint tint of color.

The tadpole is left in the stain over night, and is then run up through the increasing grades of alcohol, being finally transferred into one of the better clearing oils such as creosote or synthetic oil of wintergreen. The tadpole is then mounted in Canada balsam or dammar.

These preparations have a very great advantage over chick embryos, in the fact that no parts are missing; there is no distortion due to imperfect fixation, and there is no torsion of the body. If the preparation is properly carried out, they should be just as clear and as easily studied as are whole mounts of the chick. The brain, pituitary ingrowth, eye, auditory vesicle, pronephros, heart, stomodæum and notocord can be made out with great clearness. It is even possible to see the outlines of the cranial and spinal nerve roots.

Experience in teaching by means of these preparations has demonstrated the ease with which they can be studied and understood. It is quite possible to mount tadpoles with the dorsal surface uppermost. A special method of technique is necessary to accomplish this last named end. Either Canada balsam or dammar is heated in an oven for some days until it will harden immediately upon cooling. Heated balsam or dammar of this character is then dropped upon slides. These drops should be of the proper width, to fill the space beneath a cover slip and to at the same time maintain the desired thickness. It is not the least difficult to get drops which may be 3 or 4 millimeters in depth. They can be measured so as to give any proportion desired between breadth and depth. This can be done by heating the slide with the adherent drop over an alcohol lamp. It often chances that there are numerous small bubbles in such drops. These can be brought to the surface by placing the slides for some hours in a paraffin oven. They can then be removed by skimming the surface after heating, or in many cases they can be burst by application of a flame.

Large numbers of such slides can be prepared at a time and saved for subsequent use. They can be used, of course, for various mounts where it is desired to have the cover slip propped some distance above the slide, and in which immediate use is desirable.

Tadpoles, or other objects are mounted in the drop in the following manner: First, the drop is heated over a flame, being held there a moment or so inverted above it; then with a scalpel, previously dipped in a solvent such as xylol, a groove is made in it. This can be formed to suit the size and shape of the object to be mounted. The object is then placed in it—the sides of the groove pressed into contact with it in such a way as to hold it in the desired position. Then the space about the object is filled with a drop of soft balsam, and a cover slip, after being previously heated, is pressed down gently but firmly upon the surface of the drop. Care must be taken to prevent the inclusion of bubbles at this stage. A little practice will enable one to do this without difficulty.

The preparation should be laid aside for a week or more, and then examined. In some instances it will be found that the objects have shifted somewhat in position. They can be adjusted by simply pushing the cover slip toward one or the other side. In this way the finest adjustments in position can be made. Objects can be brought from a very decided inclination to an upright position.

Preparations of tadpoles made in this manner are especially instructive in sowing the ventricles of the brain, the eye, the developing semi-circular canals of the ear, and the different portions of the alimentary tract. It is, of course, possible to make any desired dissections of embryos, and to mount them in this fashion. I found that preparations of the pronephros, mesonephros, and developing gonads can be handled very nicely in this manner. This use of hard balsam for mounting is not radically new, but is capable of much wider use than has ever been made of it.

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CONTENTS:

WAX RECONSTRUCTION OF THE BRAIN OF AN EMBRYO LIZARD, EUMECES, ... *Clarence L. Turner.*

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Wax Reconstruction of Brain of an Embryo Lizard (*Eumeces.*)

BY CLARENCE L. TURNER.

Plates XXVIII-XXXI.

[Contribution from the Zoölogical Laboratory No. 211.]

INTRODUCTION.

THIS investigation was done with a view to comparing the general features of an embryonic brain with those of an adult lizard brain. The dissection of so small a brain was impractical, so the reconstruction method was used. No attempt was made to study the brain in connection with the skull and the other structures, although considerable information was gathered concerning them during the course of the work. The brain, with the cranial nerves and other appending structures, has been studied by itself, especial attention being given to the shape and relative position of the different lobes, the character of the cavities, the points of attachment, and to some degree the extent of the cranial nerves and to the changes in shape and position of parts that must occur between this stage and the adult stage of the brain.

The work was begun at the Ohio Wesleyan University during the year of 1912-'13, under the supervision of Prof. E. L. Rice of that university, and completed in the University of Kansas.

MATERIALS AND METHOD.

A set of slides kindly loaned by Doctor Rice furnished the material for the work. The slides contained a complete series of sections of the head of an *Eumeces* embryo, which had the following measurements: From tip of snout to posterior end of tympanum, 4.75 mm. From posterior end of tympanum to anterior end of shoulder, 2.25 mm. From anterior end of shoulder to anus, 7.50 mm. From anus to tip of tail, 13.50 mm.

The embryo had been fixed in picro-acetic acid and the sections stained with Mallory's triple connective tissue stain which brought out the brain and nerves as purple against the red, orange and blue of the other tissues.

The model was constructed according to the directions of Karl Peter in his "die Methoden der Rekonstruktion." The method is briefly as follows: Projections are made of each consecutive section by means of the stereoscope and outline drawings made of the parts to be reconstructed. The magnification is governed by the distance between the stereoscope and the screen upon which the drawings are made. In this particular piece of work a magnification of fifty diameters was used. Wax plates are next prepared, the drawings being fixed to them while the wax is still hot. The proper thickness of the plates is obtained by multiplying the actual length of the object to be reconstructed by the magnification and dividing the result by the number of sections contained in the series. Brass strips of the correct thickness are placed upon a smooth surface, the liquid wax poured upon the surface between them and a hot roller applied to the cooling wax, which is pressed out to the thickness of the strips. The plates are then cut along the lines of the drawings and are placed upon each other to form the model. In order to facilitate the building process a guide line is used. This line is introduced into the paraffin block by painting with lampblack one surface parallel to the object to be sectioned and recoating with a thin coat of paraffin. Cross sections of the tissue will then contain also cross sections of this painted surface in a constant relation to the tissue.

GENERAL TOPOGRAPHY OF BRAIN.

The plates and their explanations illustrate nearly all the points intended to be brought out, so the discussion of them will be brief. The slender olfactory lobes (A) extend posteriorly and unite with the larger cerebral lobes (B) by the olfactory peduncle.

Immediately beneath the cerebral lobes is the median optic thalami (C). At the base of the anterior margin of the optic thalami is the infundibulum (Inf), which appears as a small lobe separated from the optic thalami by a shallow constriction. The hypophysis is situated directly beneath the infundibulum, and in this stage its diameter is about half of that of the infundibulum. The pineal apparatus (H) is attached to the optic thalami between the lobes of the cerebrum and extends backward and upward from the point of attachment, lying at an angle of about thirty degrees from the horizontal plane. The parietal eye lies just above the posterior end of the pineal body.

The optic lobes (D) lie at an angle of about thirty-five degrees from the horizontal and are connected anteriorly to the optic thalami and ventrally and anteriorly to the medulla. A shallow vertical groove traverses the optic thalami at its posterior end and roughly separates it from the optic lobes.

The cerebellum (E) is relatively very small. It occurs as a median lobe at the base of the optic lobes with its long axis approximately parallel to the axis of the optic lobes.

The medulla (F) occurs directly under the division between the optic thalami and the optic lobes. It extends forward for a short distance, and then turning sharply backward it makes a U-shaped loop. The ventral horn of the loop unites posteriorly with the spinal cord (G), which bends sharply downward. Between the horns of the loop is the forward extension of the cerebellum.

CAVITIES OF THE BRAIN.

The cavities of the olfactory lobes (olfactory ventricles) appear as mere slits which twist spirally through an angle of nearly 180 degrees between their anterior end and the olfactory peduncle.

The cavities within the peduncles themselves are represented by mere lines, the walls lying in apposition.

The cavities of the cerebral lobes (first and second ventricles) are also represented for the most part as curved slits, there being very little actual free space between the walls. Fig. 7 shows the real nature of the cavities, while fig. 9 shows their vertical and fig. 8 their horizontal limits. The cerebral cavities connect with the cavity of the optic thalami through paired openings called the foramen of Monro. The opening is surrounded and partially filled by a plexus of slender, fingerlike processes.

The third ventricle is a thin vertical slit in the middle of the optic thalami. (Fig. 6, 3V.) It extends ventrally into the infundibulum and posteriorly into the optic lobes. A slender cavity also extends back into the pineal region. (Fig. 10.) The walls of the optic thalami are relatively thick.

The optic ventricles are continuations of the third ventricle into the optic lobes. They correspond in shape to the rounded external lines of the optic lobes.

The cavity occupying the medulla, the fourth ventricle (fig. 9, 4V), is joined dorsally to the union of the third and optic ventricles. A plexus extends into it from the base of the cerebellum and from the side walls, partially dividing it from the cavity of the cerebellum. (Fig. 9, P; fig. 5, P.) The posterior wall of the cerebellum is extremely thin, and it seems that a great deal of readjustment takes place at this point as the embryo passes into the adult condition.

At the base of the cerebellum the fourth ventricle opens to the exterior in the fossa rhomboidalis. (Fig. 9, I; fig 5, I.) The spinal canal joins the posterior end of the fourth ventricle.

The cavity of the hypophysis is horizontally disk-shaped, and in this stage is not connected with the main cavity of the brain.

The pineal apparatus also contains a cavity which does not connect with the main cavity of the brain. It is peculiar in having a very irregular shape and in possessing a number of blind pockets, which may be seen in the diagrams of both the vertical and horizontal planes.

CRANIAL NERVES.

The cranial nerves with their points of attachment and their extensions as far as modeled are shown in the plates, so that little more need be said concerning them.

The crossing of the nerve tracts in the optic chiasma is shown in a series of drawings (fig. 12) and a diagram representing the mode of crossing of the nerves is also represented (fig. 13). The chiasma of *Eumeces* is almost identical in its mode of crossing with *Lacerta,* the European lizard.

COMPARISON OF THE ADULT AND EMBRYONIC BRAIN.

A glance at the shape of the embryonic brain shows that a great many changes must take place in its shape as well as in its size before it reaches the adult condition. (Figs. 14 and 15.) The most evident changes are shown in fig. 15 by arrows. The tendency of the whole brain is to assume a longer axis.

The optic thalami shows a tendency to elongate anteriorly. The hypophysis is pulled back from a position directly beneath the infundibulum until it assumes a position behind and a level with the infundibulum.

The point of attachment of the sixth cranial nerve serves as an excellent mark to follow in tracing the change of position of the medulla. In the development to the adult stage the loop in the medulla becomes straightened out and the point of attachment of the sixth cranial nerve is pulled back from a position beneath the optic thalami to occupy one posterior to the optic lobes.

The spinal cord comes to lie in a horizontal plane, whereas in the embryo it bends sharply downward from its junction with the brain.

The cerebellum, which in the embryo is small and undeveloped and appears "pinched" between the optic lobes and the spinal cord, develops and takes a dorsal rather than a posterior position in reference to the rest of the brain.

The pineal apparatus extends forward from its attachment in the adult stage, while in the embryonic stage its extends backward. From the stage modeled to the adult stage it must revolve forward through an angle of about 110 degrees.

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CONTENTS:

A Study of the Dimensions of the Chromosomes of the Somatic Cells of Ambystoma, ... *Jas. B. Mack.*

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A Study of the Dimensions of the Chromosomes of the Somatic Cells of Ambystoma.

JAS. B. MACK.

Contribution from the Zoölogical Laboratory No. 212.

[Submitted as a partial fulfillment of the requirements for the degree of Master of Arts.]

FOR several years past cytologists have devoted much time to the study of the chromosomes of the germ cells, the most thorough work having been done upon the Insecta, while some has been done upon the Amphibia. But to my knowledge scarcely any investigation has been attempted upon the chromosomes of somatic cells.

It is the purpose of this paper to present some results obtained from the study of the somatic cells of Ambystoma in an attempt to discover the relation between the chromosomes of cells of the derivatives of the mesoblast and those of the ectoblast. The work along this line was suggested to the author by Dr. C. E. McClung and we have hoped that we might obtain some data which might aid us toward the establishment of a correlation of individual chromosomes and definite somatic characters.

Since the work was begun, however, a paper has appeared by Capt. C. F. U. Meek on "A Metrical Analysis of Chromosome Complexes" in which he draws the conclusion that chromosomes of different phyla possess diameters of constant size, and all those above Nemathelminthia have a diameter of .83μ, and that the somatic chromosomes are identical with those of the germ cells. This has led me to conduct my investigations along a somewhat different line from that which I originally intended in order to corroborate his statements if possible. To make the arguments still more conclusive, in addition to the work upon Ambystoma, I made a few measurements and drawings of the chromosomes of Gryllus. If Meek's theory can be established as a law which will hold throughout the animal kingdom it will be of inestimable value.

MATERIAL AND METHODS.

The material for the study of both *Ambystoma* and *Gryllus domesticus* was prepared in the laboratories of Kansas University. The Ambystoma was fixed in Flemming's Fluid and stained with Heidenhain's iron-hæmatoxylin. The slides for the Gryllus were kindly loaned me by Prof. W. J. Baumgartner. These were all fixed in Flemming's fluid; some were stained with iron-hæmatoxylin and some with Flemming's triple stain.

The preparations were studied by means of a Leitz apochromatic oil immersion $\frac{1}{12}$ a objective and a No. 4 ocular and a No. 18 compensating eye piece. The source of illumination for most of the work, and especially for the measurements and drawings was ordinary daylight projected upon the mirror of the microscope by means of a funnel made of heavy black paper inserted in other heavy paper which covered the greater part of the window, thus admitting only a small amount of light between the shade and the heavy paper. Part of the work was done by gas light with a Welsbach burner, the rays passing through a copper sulphate solution.

The drawing board was built on a level with the microscope stage. The measurements and drawings were made by the aid of an Abbe camera lucida, so adjusted that the center of each measurement and each drawing was 100 mm. from the center of the objective. The measurements were all made with the draw tube adjusted so that the distance from the drawing table to the upper part of the ocular was 231 mm., thus giving a magnification of 4000 diameters. The drawings were made with the draw tube adjusted so that the distance was 211 mm., giving a magnification of 3200 diameters. The drawings represented in the plate here are reproduced at 2145 diameters. The magnifications were determined by means of a Zeiss stage micrometer.

OBSERVATIONS.

It will not be necessary to go into details concerning the early prophase of mitosis as that has already been dealt with by other investigators. By a count of the chromatin knots and the chromosomes of the telophase, and especially of the metaphase, I find the number to be 24 in the cells of the various tissues, agreeing with the number in the spermatogonial and primary spermatocyte as given by the *Schreiners and Meek. Baumgartner seemed to discover a difference in the form of the chromosomes; Sutton a difference in size; and Meek a difference in measurements. I wish to apply these tests to the somatic cells to determine whether by a study of the form, size and dimensions we can arrive at some definite conclusions concerning their relation to the germ cells.

I made a careful study of several hundred cells, and made measurements of the chromosomes of most of them, and compared those of the neuroplasm with those of the ectoplasm, the muscle, the connective tissue and the pigment tissue. In all cases I found them grouped about the equatorial plate in an irregular manner. They are always in the U or V shape with the spindle fibres attached one at the vertex of each chromosome. These fibres are almost invariably attached in such a way that the two arms of the V are of unequal length.

As will be seen in figs. 4 and 5 the chromosomes in the same cell are often irregular in outline, thick in some places and thin in others. The chromosomes of the connective and muscular tissue, while resembling each other in form and size, have some differences and are not like those of other tissues. Those of the muscular tissue are rough and short, while those of the connective tissue are short, thick and smooth. Those of the pigment cells are always very smooth and also much longer in proportion to their breadth and can invariably be distinguished by their high staining qualities, always being a bright violet in the preparations studied.

Some of the chromosomes differ in size because of difference in tissue and also because of the difference in the age of the tissue, as will be brought out more definitely a little later. They also differ in size in individual cells but the difference is so slight in any one cell that it is difficult to determine without accurate measurements, neither is it possible to arrange them in any such system as some authors have done, of three small, two medium and five long ones, etc. Without a doubt they have an individuality and "these various shapes are an expression of the individual characteristics of the various chromosomes" as †Baum

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* A. and K. E. Schreiner. Neue Studien uber die Chromatinreifung der Geschlechtszellen.

†Baumgartner ('04). Some New Evidence for the Individuality of the Chromosomes.

gartner ('04) has stated. But there is not sufficient difference either in form or size to substantiate a theory of persistence of this individuality.

In studying the size of the chromosomes more carefully I made sketches of from five to eighteen chromosomes in each of fifty cells in the Neuroplasm, 21 cells in the ectoplasm, 15 cells in the muscle, 15 cells in the connective and 10 cells in the pigment. I did this for each of the three different ages of the Ambystoma, using the camera lucida and micrometer, as described previously. The accompanying table gives a record of the results obtained. For convenience we will use the series number of the slide 628 to represent the youngest and 630 the oldest.

No. of slide.	No. cells.	Tissue.	Av. Width.	Metaphase.	Anaphase.	Extremes.
			μ	μ	μ	μ μ
628	50	Nerve	.87	.85	.77	.65 to 1.02
	21	Ectoplasm	.83	.82	.77	.55 to 1.10
	15	Muscle	.663	.67	.67	.63 to .83
	10	Pigment	.725	.715	.70	.70 to .80
	15	Connective	.767	.73	.752	.65 to .98
629	50	Nerve	.797	.83	.71	.60 to 1.05
	21	Ectoplasm	.775	.79	.73	.63 to 1.20
	15	Muscle	.80	.78	.50	.50 to 1.08
	10	Pigment	.85	.75	.73	.65 to 1.30
	15	Connective	.735	.80	.65	.55 to 1.20
630	50	Nerve	.75	.80	.65	.53 to 1.70
	21	Ectoplasm	.75	.75	.68	.57 to 1.10
	15	Muscle	.70	.68	.53	.48 to .93
	10	Pigment	.70	.68	.73	.53 to .92
	15	Connective	.65	.73	.70	.58 to .97

Great care was taken to use only chromosomes in which the complex lay perpendicular to the line of sight lest there be a possibility of foreshortening. In many cases it was possible to find a complex, or nearly a complete complex, in which the diameter was uniform, and in which the length seemed to form some kind of a mathematical series if not an arithmetical one. But this was the exception rather than the rule. We know that the larger the number entering into an average, the less is the liability of error, and the more general and reliable becomes our deduction. For this reason I tested 50 nerve cells instead of one or two. I measured the diameter of from 5 to 18 chromosomes, in each of these cells and worked out the average for each cell and then the average for the entire 50 nerve cells. We find by referring to the table here for No. 628 that the average width is .87 μ. The average for the metaphase and anaphase alone are .85 μ and .77 μ respectively. But in the individual cells there is a greater difference. The averages for the cells range from .65 to

1.02 μ. If we compare these figures with the corresponding results recorded for the nerve tissue of Nos. 629 and 630 we notice a marked difference in the diameter, which seems to decrease as the age increases. This holds true with those of the different phases as well. The variation in the averages continues very great. In No. 630 it ranges from .53 μ to 1.70 μ. The cells showing mitosis are far more numerous in No. 628 than in the others and the amount of nerve tissue is greater also as might be expected. The results for the other tissues were worked out in a similar manner. The number of cells showing mitosis in the other tissues is much less than in the nerve tissue, so that 21, 15 and 10 were about all the good specimens that could be found in these different tissues.

In the ectoplasm for No. 628 the general average is .83 μ, for the metaphase alone it is .82 μ with various averages for individual cells ranging from .55 μ to 1.10 μ. If we compare these figures with those for the chromosomes of the ectoplasm of No. 629 and No. 630 we find in one case the average is .775 μ and in the other .75 μ and for the metaphase alone it is .79 μ and .75 μ, with still greater extremes in the general averages ranging from .63 μ to 1.20 μ in one case and .57 μ to 1.10 μ in the other. As in the nerve plasm the diameter decreases as age increases.

In the muscle the average for No. 628 is .663 μ and for the metaphase .67 μ with a variation in extremes of only .63 μ to 83 μ. Contrary to the action of the chromosomes in the nerve plasm and ectoplasm, the general average of diameters increases in No. 629 to .80 μ and .78 μ for metaphase and then decreases slightly in No. 630 to .70μ for the general average and .68 μ for the metaphase, with great variations and extremes.

The chromosomes of the pigment cells have an average diameter in No. 628 of .725 μ. The metaphase has a diameter of .715 μ. The chromosomes in No. 629 of the same tissue has a diameter of .85 μ, the metaphase of .75 μ. The extremes are .65 μ and 1.30 μ. The diameters in the pigment cells seems to be greatest in No. 629 and least in No. 630 both for the metaphase and for the general average, neither following the results for the nerve nor for the muscle.

Comparing the results of the pigment with those of the nerveplasm, ectoplasm and muscle we find that the diameter in No. 628 is greater than the diameter for the muscle and less than for the nerveplasm and ectoplasm. In No. 629 it is greater than in any of the others and in No. 630 it is less than any of the others except for the muscle, which it equals. Considering the metaphase alone the average is less than any of the other metaphases for No. 628, the same in No. 629 and the same in No. 630.

Considering the chromosomes of the connective tissues we find the average diameter for No. 628 to be .767 μ, for the metaphase alone it is .73 μ with the greatest diameter found in the anaphase. In No. 629 the average is .735 μ and the greatest diameter is found in the metaphase. The extremes of the averages being .55 μ and 1.20 μ. In No. 630 it is .65 μ and for the metaphase it is .73 μ, this being again above the anaphase. Here again we find the greatest diameter in the younger, and decreasing with age, but for the metaphase alone it is greatest for No. 629.

Comparing those of the connective tissue with those of the other tissue we find the total average less than for any of the others in Nos. 629 and 630 but greater than those of the muscle or pigment in No. 628.

It is quite conclusive from these figures that there is a difference in chromosome diameters due to age, and that there is a difference in different tissues of the same age, but this variation is not constant. The diameter is neither constant in all phases of the same tissue, as Meek declares, nor is it the same for any one phase in all the tissues. The diameter is not necessarily constant for all the chromosomes in any one somatic cell of any particular tissue, although it is possible this may occasionally happen. The diameters of the chromosome of the neuroplasm and the ectoplasm decrease as the age increases.

It was practically impossible to find cells where all the chromosomes of a complex lay in a plane perpendicular to the line of vision so that one could make accurate measurements of the lengths and diameters of all the chromosomes of a complex, as will be seen by noticing in Figs. 2, 3, 4, and 5 the various positions of the individual chromosomes. The dimensions of 25 chromosomes were taken and the volumes worked out to see if any constancy existed there. The ends were considered to be rounded. The two ends making a sphere whose diameter equalled the diameter of the chromosome and the intermediate space being a cylinder, whose diameter is the diameter of the rod and the length equal to the length of the chromosomes, less its diameter. The volumes ranged from 1.54 cu. μ to 17.94 cu. μ. In a single cell the lengths ranged from 7.5 μ to 10 μ, and the diameter from .63 μ to 1.00 μ and the volume from 2.84 μ to 5.63 cu. μ.

*Meek ('12) declares that the diameters of the chromosomes

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* C. F. U. Meek. A Metrical Analysis of Chromosome Complexes.

for this phyla are constant and equal to .83 μ and on page 12 states that "a study of the lengths of the ordinary chromosome rod leads to the discovery that they constitute a series in arithmetical progression the short chromosomes being consecutive members." On page 25 he asserts that "the complexes of a species and its variety appear to be identical; differences, if existing, are too small to be recognized"; also that "the somatic chromosomes are identical with those of the germ cells." He further adds that "the total volume of ordinary chromosomes is the same in the spermatogonial and primary spermatocyte metaphases, whereas only half this amount appears in that of the secondary spermatocyte." If the somatic chromosomes are identical with those of the germ cells we should naturally expect them to follow his theory concerning the spermatogonial and the first spermatocyte chromosomes in diameter, length, and volumes. From a careful study of my results previously recorded it is very evident that in no case was I able to find such a constant diameter nor such a regularity in the variation in length of the chromosomes in the material at hand.

In order to ascertain whether this variation in size was peculiar to the somatic cells only, I measured from seven to ten chromosomes in the metaphase in each of twenty-two cells of the first spermatocyte in *Gryllus domesticus,* and those of one in the anaphase, and those of two in the metaphase of the primary spermatogonian, the lengths of the chromosomes in one of the best cells of the spermatocyte were 3.5, 3.75, 2.5, 2.25, 2.25, 2.25, 2, 2, 5, and 1.5 μ. The corresponding diameters were 1.25, 1.75, 1.5, 1.75, 1, 1, 1, 1, 1.25, and 1 μ. The lengths of another were 3, 2.5, 2.25, 2.25, 2.25, 2, 2, 1.5, 1.5, and 1.25 μ, and the corresponding diameters were 1.25, 1.5, 1.5, 1.25, 1.25, 1.75, 1.5, 1.25, 1, and 1.25 μ. The lengths for one of the best spermatogonial cells were 3, 2.5, 2.5, 2.5, 2.25, 2.25, 2, and 2 μ, while the diameters were .50, .75, .75, .62, .75, .75, 1.25, and .50 μ. I could in none of these cases, as can easily be seen, find an absolute constant diameter as Meek mentions for the same animal in the same species, although there were several diameters which were identical. Nor could I find the relation between the lengths which could be called "an arithmetical series whose common difference was less than half of the diameter." In order to avoid any error which might occur, due to fixation, I was careful to choose cells which were similarly located in the testis.

In order that I might discover whether I was in error, I next chose two cells, one of the primary spermatogonial and one of the first spermatocyte as illustrated in Baumgartner's paper on *Gryllus domesticus.* In the spermatogonia the diameter was .514 μ, but not .83 μ, as Meek gives for the same material, and the lengths range from .86 to 2.41 μ, and not from .8 to 6.71 μ. In the first spermatocyte the diameter of seven was .514 μ, that of one was .69 μ and that of another was 1.04 μ, excluding the accessory; the lengths ranging from 1.04 to 4.13 μ.

SUMMARY.

The number of chromosomes is twenty-four in the somatic cells of the Ambystoma, agreeing with the number in the primary spermatocyte.

The chromosomes are grouped about the equatorial plate in a U or V shape with the spindle fibres attached, one at the vertex of each chromosome, so that the arms of the V are of unequal length.

They differ in size and form in different tissues, being thick and rough in outline in some, and long, slender and smooth in others.

The chromosomes of the pigment cells are long and thin in proportion to their length and possess higher staining qualities than the others.

It is impossible to arrange the chromosomes in any one cell in any systematic classification according to form or size.

There is a difference in chromosome diameter due to age and there is a difference in different tissues of the same age, but this variation is not constant.

The diameter is not constant in all phases of the same tissue.

The diameter is not constant for all the chromosomes in any one somatic cell of any particular tissue.

The diameters of the chromosomes in neuroplasm and ectoplasm decrease as the age of the animal increases.

The diameter and volume of the phyla are not constant, neither do their lengths constitute an arithmetical series.

The volumes range from 1.54 to 17.94 cu. μ and in a single cell the volume ranged from 2.84 to 5.63 cu. μ.

The germ cells may have several diameters which are identical but they do not posses an absolutely constant diameter, nor do the lengths form an arithmetical series.

I wish to express my appreciation to Dr. C. E. McClung for starting me on this material and to Prof. W. J. Baumgartner for suggestions and for the kindly interest shown in the work. I am also indebted to Dr. T. C. Frye for permitting me to use a room in the Puget Sound Marine Station.

Since this paper has been sent to the publisher new papers have appeared by Meek on "A Further Study of the Metotic Spindle in the Spermatocytes of Forficula auricularia," in which he says that "new evidence before us shows us that the previous proposition put forward can not be universal," and that, "so far cytometrical investigations seem to have yielded only negative generalizations," thus agreeing with our results.

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Florence S. Hague.

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VOL. XIX, NO. 11

Numerical Relation of Spermatozoa to Sertoli Cells.[1]

FLORENCE S. HAGUE.

[Contribution from the Zoölogical Laboratory No. 213.]

THIS study was started with the purpose of ascertaining the number of spermatozoa associated with each Sertoli cell in the human testis. However, on account of being unable to get material which was in the right condition, the problem was changed to lower mammals.[2]

The tissues used were from the testes of man, the cat, and the rat. Nothing was known as to the age or condition, healthy or otherwise, of the human material. The pieces sectioned had been preserved in formalin. Several celloidin mounts were also studied. Flemming's tri-color, Davis' cytological stain, and iron hæmatoxylin with acid fuchsin, and eosin, as counterstains, gave the best results. Delafield's hæmatoxlyn with orange G, congoroth, and bordeaux red resulted in very lightly stained sections. For fixing the rat testes Mueller's, Gilson's, Bouin's, Tellyesniczky's and Zenker's fluids were used, the last two of which were the most satisfactory. The cat testes were preserved in Tellyesniczky's and Flemming's solutions. For sections of both the rat and cat testes tri-color, iron hæmatoxylin with or without eosin, dilute Delafield's hæmatoxylin and hæmalum were used. The sections varied in thickness from 6 to 40 micra.

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[1] I wish to acknowledge the assistance and direction of Prof. W. J. Baumgartner and Dr. B. M. Allen in this problem.

[2] The numerical relation is now being worked out in the cat. A similar study is planned for other forms.

The only statements of ratios of spermatozoa to Sertoli cells, thus far found, are those of Montgomery and Winiwarther. Both of these base their figures on the proportion—3 to 1—of spermatogonia without rods to spermatogonia with rods, or crystals. They agree that from the cells containing rods Sertoli cells develop. However, Winiwarther says that the three cells with rods develop into primary spermatocytes, while Montgomery asserts that they divide before becoming primary spermatocytes. The latter thus gets the ratio of 24 spermatozoa to one Sertoli cell, and the former the ratio of 12 spermatozoa to each Sertoli cell.

After comparatively little study of mature Sertoli cells and spermatozoa in the rat, the conclusion was reached that there could not be as many as 24 spermatozoa to each Sertoli cell. Then the theory that there are 12 spermatozoa to each Sertoli cell was assumed, but this too had to be abandoned, since the counts showed smaller numbers.

In the human material available the spermatozoa have nearly all been expelled. Accordingly, distinct Sertoli cells filled with spermatozoa are hard to find. Three cells were found in which there were twelve spermatozoa, a few in which there were ten or eleven, and more in which there were eight or nine spermatozoa. However, the writer has no way of telling whether or not the Sertoli cells are complete, for the sections are only 10 micra thick, with the exception of the celloidin ones. The few spermatids are not grouped together thus making counts impossible.

The rat testes were taken from two animals, and were in a condition of very active production of spermatozoa. In the rat the mature Sertoli cells are long and slender, and have the appearance of striations. The spermatocytes between the Sertoli cells are small and identical. Consequently tracing a Sertoli cell from one section to another is extremely difficult. Sections varying in thickness from 6 to 24 micra were stained lightly with Delafield's hæmatoxylin or hæmalum. The spermatozoa, however, took the stain so lightly that they can not be distinguished when packed in the cells. Sections 15 micra thick stained with iron hæmatoxlyin are so much better, that some counts were made from them, also from a section of tissue preserved in Flemming's fluid, but which was already prepared. Thinking that there were twelve spermatozoa in each Sertoli cell, and that the cells might not always be complete, only the larger numbers were tabulated. The following are the counts:

11 Sertoli cells with 12 spermatozoa.
18 Sertoli cells with 11 spermatozoa.
20 Sertoli cells with 10 spermatozoa.

In another effort to trace Sertoli cells from one section to another, several successive cross sections of tubules of 15-micra sections were outlined, and the groups of spermatozoa were located and counted. Even though there was no success in tracing the cells, there were other results from these counts. Since Sertoli cells are about 50 micra long and 12 micra thick when fully developed, there should be in sections 15 micra thick, some complete cells, or cells with 12 spermatozoa, about equal numbers of cells with 11 and 1, with 10 and 2, with 9 and 3, with 8 and 4, with 7 and 5, and about twice as many with 6 spermatozoa, providing there are 12 spermatozoa in each Sertoli cell. But the following are the numbers in 6 sections:

14 groups of 1 each.
26 groups of 2 each.
32 groups of 3 each.
32 groups of 4 each.
57 groups of 5 each.
70 groups of 6 each.
65 groups of 7 each.
35 groups of 8 each.
13 groups of 9 each.
6 groups of 10 each.
2 groups of 11 each.
3 groups of 12 each.

Since this did not give any definite ratio, groups in eight sections 8 micra thick were counted, with the following results:

29 groups of 1 each.
24 groups of 2 each.
27 groups of 3 each.
26 groups of 4 each.
49 groups of 5 each.
44 groups of 6 each.
41 groups of 7 each.
21 groups of 8 each.
6 groups of 9 each.
3 groups of 10 each.
0 group of 11
1 group of 12

In the first of these counts the sections are thick enough so that one Sertoli cell would not be in more than two sections, but the numbers of groups of 1, 2, and 3 spermatozoa far surpassed the numbers of groups of 9, 10, and 11. From this it seems that the number can not always be as high as twelve, and seldom, if ever, higher. In the 8-micra sections, it would be possible for a Sertoli cell to be in three sections, and this would account for a larger number of small groups of spermatozoa, but hardly for as great a difference as there is. In both counts the numbers of groups of 5, 6, and 7, are far in excess of other numbers.

Short pieces of unstained tubules were teased and stained. Some pieces of tubules were stained in alum cochineal, or Delafield's hæmatoxylin, cleared in cresote and then teased or cut longitudinally and spread out. One difficulty here is being unable to determine the stage of development of the spermatozoa. It is quite seldom, too, that the Sertoli cells are sufficiently isolated to enable one to make counts of their contents. No counts were recorded, but in those cells which seem to be complete there are more often 8, 9, or 10 spermatozoa than 11 or 12.

Finally, sections 20, 25, 30, 35, and 40 micra thick were prepared and stained with iron hæmatoxylin with or without eosin, and with tricolor. The tricolor did not give as good results, though less destaining would probably have improved it. With iron hæmatoxylin, the spermatogonia, spermatocytes and spermatids are all a light brown, and the spermatozoa a dead black when mature. Thus especially with an immersion oil objective, the spermatozoa are clear and distinct. In the 20-micra sections a few Sertoli cells can be found, the spermatozoa of which are invisible at both the upper and lower plane of the section. In sections 30 micra thick a Sertoli cell with 7 or 8 spermatozoa may be in focus at the upper level of the section, may disappear, and at the lower level another cell immediately beneath the first and equally well filled may come into focus. In 40-micra sections a third cell may sometimes be seen directly above two others. Thus there can be no doubt but what complete cells are contained in these sections.

In all counts care was taken to have the complete thickness of the cell, and in practically all the entire length could also be traced. Counts were made in several stages. The clearest and easiest were those made when the spermatozoa were approaching and at the base of the cell, or at the base and beginning to move outward. In both these conditions each Sertoli cell is almost invariably completely surrounded by spermatocytes. In the latter case sections of tubules with the lengths of the lumens extending across the entire field, with the low power, were used and no spermatozoa were seen in the lumens, or had even reached the inner portion of the Sertoli cells. Counts of very early stages of spermatozoa were very difficult, because they are less clearly outlined, and the Sertoli cells are thicker, more branching, and come in contact with each other about the lumen, thus making the groups of immature spermatozoa less distinct than the mature groups. The spermatids are not sufficiently grouped to give reliable accounts.

In all the following counts the microscope was carefully focused each time to see that the cell was complete. First mature spermatozoa were counted in longitudinal sections of tubules, in the lumens of which there were no spermatozoa; neither had the spermatozoa advanced to the inner portion of the Sertoli cells. Thus none can have escaped. The following groups of spermatozoa in Sertoli cells were counted in sections 30, 35, and 40 micra thick:

10 groups of 6 spermatozoa.
30 groups of 7 spermatozoa.
45 groups of 8 spermatozoa.
35 groups of 9 spermatozoa.
3 groups of 10 spermatozoa.

In sections of the same thickness groups of spermatozoa which had just reached or were approaching the bases of the Sertoli cells were counted, and the following results obtained:

9 groups of 6 spermatozoa.
26 groups of 7 spermatozoa.
24 groups of 8 spermatozoa.
16 groups of 9 spermtaozoa.
5 groups of 10 spermatozoa.
4 groups of 11 spermatozoa.

Finally very early spermatozoa were counted in sections 40 micra thick, most of which were stained with iron hæmatoxylin and counterstained with eosin. The counts of these immature spermatozoa are as follows:

10 groups of 6 spermatozoa.
23 groups of 7 spermatozoa.
27 groups of 8 spermatozoa.
25 groups of 9 spermatozoa.
10 groups of 10 spermatozoa.
2 groups of 11 spermatozoa.
1 group of 12 spermatozoa.

The total of 313 counts from sections 30, 35, and 40 micra thick are:

29 groups of 6 spermatozoa.
77 groups of 7 spermatozoa.
106 groups of 8 spermatozoa.
76 groups of 9 spermatozoa.
18 groups of 10 spermatozoa.
6 groups of 11 spermatozoa.
1 group of 12 spermatozoa.

In two cells which appeared to be whole only 5 spermatozoa could be made out, and a few times, especially among immature spermatozoa, groups were so close together as at first to appear as one, thus forming groups of more than twelve. It is possible that groups of 5 or 13 or 14 do occur, but if so, they are very rare. From these counts and the earlier ones it is evident that the number of spermatozoa to each Sertoli cell in the rat varies from 6 to 12 and is oftener 7, 8 or 9.

The rod or crystal which Montgomery and Winiwarther describe is not visible in the human material studied, but this can doubtless be accounted for in the fixation. The Sertoli cell nuclei stain darkly and are always irregularly shaped and distributed. The Sertoli cells of the cat testes on the contrary do show rods and rodlets, in the tissue preserved in Tellyesniczky's fluid as well as that preserved in Flemming's fluid. These rods are, presumably, structures similar to those of human Sertoli cells for they are long and slender, sometimes curved, and irregularly placed. They are, however, not as distinct as those of the human testes as illustrated by Montgomery and Winiwarther. It is only with very careful focusing that they can be seen at all, and a few times this focusing has made them appear to be corners or edges of the nuclear membrane. With their irregularly shaped nuclei this might indeed be possible. Winiwarther, although, he calls these structures crystals, and has discussed them at some length, has been unable to prove their crystalline properties by polarization of light and even says that he has never been able to identify with certainty these crystals of Charcot. Besides the rod or crystal, both authors describe rodlets or accessory strips (batônnets), which Montgomery says persist longer, while Winiwarther says the rods persist after the rodlets disappear. The rods have been seen after the Sertoli nuclei were well pushed away from the wall of the cat testes.

None of the mature Sertoli cells of the rat testes show either rods or rodlets. About the periphery of a section preserved in Flemming's fluid and stained with iron hæmatoxylin, there are occasional dark streaks on the Sertoli nuclei, which at first appeared to be rods. These are not visible in the lighter cells of the central part of the section. The nuclei are uniformly slightly granular and contain a large nucleolus and two or three smaller chromatic bodies, one always close to or attached to the nucleolus.

In the human testis by far the greater part of the cells seen were primary spermatocytes in various stages, but none dividing. At the periphery there were also a few cells in earlier stages, and at the lumen some spermatids and spermatozoa. Some sections of tubules of the rat testis show a row of developing primary spermatocytes at the periphery, then secondary spermatocytes, between the long Sertoli cells which contain mature spermatozoa. Other tubules show the primary spermatocytes almost mature, secondary spermatocytes, and Sertoli cells with spermatozoa, pulling away from the wall of the tubule and passing toward the lumen. At the same time there can be seen in other tubules a row of the large primary spermatocytes inclosing a mass of secondary spermatocytes, which in turn enclose the last of the escaping spermatozoa. The secondary spermatocytes divide quickly into spermatids and then first begin to form in groups. When they are seen elongated and in definite groups about the inner ends of the Sertoli cells, which have grown inward, the mature primary spermatocytes are dividing and the next generation is beginning to appear. The spermatozoa begin to form at the periphery of the lumen, but as they condense more and as the secondary spermatocytes which are being formed, push inward, the spermatozoa make their way toward the base of the Sertoli cell, often clustering close about the nucleus. Even when passing out the nucleus is frequently very close to the spermatozoa.

Montgomery in speaking of ultimate spermatogonia one quarter of which, he says, contain rods and three quarters lack rods, says: "The ratio is somewhat less than 1 to 3, which is readily explained on the ground that some of the spermatogonia with rods had already become Sertoli cells and therefore were not included in the count." It would seem, then, that Sertoli cells develop from the ultimate spermatogonial condition before the other three cells divide and develop into the primary spermatocytes. Then, assuming that the principles of spermatogenesis in the rat are the same as those Montgomery describes for man, there ought to be Sertoli cells, perhaps not quite mature, for each generation of germ cells in the tubule. Usually three generations of germ cells—primary and secondary spermatocytes, and spermatozoa—are distinctly visible in the tubules. But only when one generation of spermatozoa and Sertoli cells are passing out can a second generation of Sertoli cells be distinguished. Where, then, are the successive quarters of ultimate spermatogonia which contain the rod, some of which develop into Sertoli cells before the other three quarters of ultimate spermatogonia can divide? It would seem that they would at least be visible by the time the other three quarters had become secondary spermatocytes.

CONCLUSIONS.

1. The Sertoli cells of the rat do not show a rod-like structure, but those of the cat do.
2. There is no definite connection between Sertoli cells and germ cells until the spermatozoa are beginning to form.
3. The number of spermatozoa to each Sertoli cell varies from 6 to 12 in the rat.

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THE

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(Whole Series, Vol. XIX, No. 12.)

CONTENTS:

STUDIES UPON THE NUMBER OF GERM CELLS OF CERTAIN FISH, ... *Nellie Taylor, Cora M. Downs.*

PUBLISHED BY THE UNIVERSITY,
LAWRENCE, KAN.

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Vol. IX, No. 12.] DECEMBER, 1914. [Whole Series Vol. XIX, No. 12

Studies Upon the Number of Germ Cells of Certain Fish.

Fundulus heteroclitis.—Nellie Taylor.
Embiotica jacksoni.—Cora M. Downs.

[Contribution from the Zoölogical Laboratory No. 214.]

Explanatory Note.—B. M. Allen.

The purpose of the following papers is to show the typical number of germ cells of certain vertebrates during certain early stages in development in which there is for a long period no multiplication. It is believed that this is a matter of considerable theoretical interest. Each species of vertebrates has a typical average number of germ cells present at this time, and it is thought that future investigation may perhaps show that the final number of germ cells produced in a yield will prove to be some multiple of this. Upon such an assumption there is considerable interest in comparing the number of germ cells in different individuals. This work was done under my supervision and I feel full confidence in the results given below. This work is in line with similar counts made by Eigemann, Beard and by myself upon various forms of vertebrates. These counts of the germ cells of these teleosts are of considerable interest. This is especially true in the case of *Fundulus heteroclitis*, a form that has served for so much valuable work along experimental lines.

The eggs and embryos of *Fundulus heteroclitis* were collected at Woods Hole, Mass. They were preserved and fixed in Zenker's fluid and 5 per cent formalin.

The material of *Embiotoca jacksoni* was gathered at San Diego, Calif., fixed in bichromate-acetic fluid and preserved in 70 per cent alcohol.

Paraffin sections were cut the thickness of 10 micra, and stained in iron-hæmatoxylin and orange G. Great care was

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taken to repeat these counts, and there is every reason to believe that they are approximately correct within a very short margin of error.

Table showing number of sex-cells in embryos varying in size from a total length of 3 mm. to 7 mm.:

FUNDULUS HETEROCLITIS.

Age, 24 days.

Number.	Length.	No. of cells.	Av. No. cells.
1	6 mm.	24	..
2	6½ mm.	27	..
3	7 mm.	32	..
4	7 mm.	35	..
5	6½ mm.	43	32

Age, 17 days, varying in length from 5 mm. to 6½ mm.

Number.	Length.	No. of cells.	Av. No. cells.
1	5 mm.	32	..
2	6 mm.	29	..
3	6½ mm.	41	..
4	6½ mm.	45	37

Age, 7 days, varying from 3 to 4 mm. in length.

Number.	Length.	No. of cells.	Av. No. cells.
1	3 mm.	30	..
2	3½ mm.	36	..
3	4 mm.	40	..
4	4 mm.	38	..
5	3⅓ mm.	37	36

EMBIOTOCA JACKSONI.

Table showing the number of primitive germ cells in embryos varying in size from 7.58 mm. to 3.25 mm.:

Stage.	Fish.	No. of cells.	Average.
7.58 mm.	A	8	..
	B	12	..
	C	10	..
	D	10	..
	E	10	10
4.96 mm.	A	15	..
	B	7	..
	C	9	10.3
4.76 mm.	A	15	...
	B	18	...
	C	13	...
	D	13	...
	E	9	13.6
3.36 mm.	A	12	12
3.25 mm.	A	9	9

THE
KANSAS UNIVERSITY
SCIENCE BULLETIN.

Vol. IX, No. 13—December, 1914.

(Whole Series, Vol. XIX, No. 13.)

CONTENTS:

A Preliminary Report on the Infusoria of Kansas, *Inez Frances Smith.*

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VOL. IX, NO. 13.] DECEMBER, 1914. [WHOLE SERIES
VOL. XIX, NO. 13

A Preliminary Report on the Infusoria of Kansas.

BY INEZ FRANCES SMITH.

[Contribution from the Zoölogical Laboratory, No. 215.]

Plates XXXIII-XLIX.

INTRODUCTION.

IN connection with a movement which was begun in the University of Kansas last year to secure, through the coöperation of advanced students, a survey and classification of Kansas Protozoa, I have undertaken to draw and classify some of the common species of Infusoria occurring in the state. Another portion of this survey, *Shelled Rhizopods of Kansas*, by Miss Regina Woodruff (to be published in the *Kansas Science Bulletin*), is, at the time of this writing, in the publishers' hands. These reports will be followed by others upon the other classes of Protozoa, or upon as yet untouched members of the classes already undertaken. As it was not intended that this survey, or any very considerable portion of it, should be the work of one student, and as at present time will not permit me to carry my work further, it was thought best for me to publish this preliminary report now.

The class upon which I have undertaken this work is a large one, and it is self-evident that I have made no very thorough survey of the field. On the other hand, I am confident that, during even the short time in which I have been engaged in this task, I have been compelled by lack of time to pass by fully half of the species of Infusoria that have come under my notice. So numerous are these species that it has not been necessary for me to search for material in other parts of the state. The species included in this report, therefore, are those found in Lawrence, Kan., or its immediate environs.

In working with Infusoria one soon learns that his classification and most of his drawing must be done from the living animal, for, when killed, nearly all species of Infusoria become abnormally distorted and lose all evidence of ciliation. Even the best-preserved species are unreliable. My method with those forms which do not become distorted when killed has been to make a free-hand drawing of the animal when alive, showing all of the details then visible, and a second camera-lucida drawing of the animal after it has been fixed and stained, showing the outline of the body, of the nuclei, and of any other structures that have been sufficiently well preserved. The two drawings were then used to supplement each other in the finished plate. In a great many cases I have been able to keep the specimen under observation quiet for a sufficient length of time to enable me to get a hasty camera-lucida sketch of the living animal. This method was necessary in dealing with those animals which contract or become greatly distorted when killed, and was used whenever possible. In a few cases, chiefly with large forms like *Spirostomum,* I was compelled to construct the drawings from measurements taken.

The fixatives and stains used were, for the most part, those in general use for Protozoa; and the fixative or stain best adapted to a particular form had to be decided upon by experimenting. I have found an aqueous solution of corrosive sublimate plus 5 per cent acetic acid very useful in killing some easily distorted forms. Schneider's aceto-carmine has proved to be a very convenient, quick, combined fixative and stain where delicate cytological mounts are not required. This fluid kills many Infusoria in better condition than any other I have used.

One of the chief difficulties in dealing with Infusoria is to get them sufficiently quiet to make out the structural details. In this a weak solution of tannin in distilled water served me best. It worked especially well with Hypotrichs where the ciliation is not only difficult to make out but very important in classification. The careful application of a solution of tannin served to bring the animal almost to a standstill, while the cilia moved vigorously.

In classifying these forms, I have attempted to take into consideration the great variation in size which such species are liable to undergo. It is a well-known fact that different races of Paramæcia vary to a marked degree in size, and it is also well known that after one or two rapid divisions a protozoan is reduced to one-half or one-fourth of its original bulk. It is therefore, I believe, quite as absurd to attach too much importance to size in classification of Protozoa as it would be to declare a child not human because it is not so large as an adult. That does not mean, however, that size is to be entirely neglected, and as it is often convenient for the student to know something of the variation in size of certain species I have included, wherever expedient, not only the size of the specimen at hand but also the range in size, or average size, of the species, as indicated from a comparison of the work of various writers.

All drawings, with the exception of two plates of Heterotrichs and a few fully extended forms or entire colonies, have been made to the same scale, for convenience in rapid comparisons.

A key has not been included in this report, because that was deemed unnecessary until a more complete survey of the field shall be made. The classification is, in general, that of Calkins.

I have made no attempt to create new genera or species, because, unfortunately, I have been unable to collect all of the literature upon the subject. I have, therefore, omitted for the present most of the forms which I have been unable to classify.

The species contained in this report have been taken from a variety of sources, including: hay and leaf infusions, water from ponds, surface pools, small creeks, watering troughs and wells.

In concluding this introduction, I take pleasure in acknowledging my indebtedness to Miss Nadine Nowlin, under whose direction the work was done, for the time and invaluable assistance which was given whenever and wherever it was needed.

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CLASSIFICATION AND DESCRIPTION.

CLASS INFUSORIA.

The term Infusoria is applied to one of the four large classes of Protozoa. To this class belong those Protozoa having cilia (1) during the entire life cycle, or (2) during embryonic life only.

Subclass CILIATA.

Infusoria which retain their cilia during the entire life cycle.

Order HOLOTRICHA.

Ciliata in which the cilia are universally distributed over the cuticular surface of the body and are all approximately equal in length and thickness.

Suborder GYMNOSTOMINA.

Holotricha with mouth which can be closed. Undulating membrane lacking.

Family ENCHELINIDÆ.

The mouth which can be closed is terminal or subterminal. Food is taken in by swallowing.

Genus *Coleps* Ehrenberg.

Body persistent in form, more or less barrel-shaped, but not always symmetrical. Surface covered by an armor of plates arranged around the body in zones with cilia located in the intervening furrows. Mouth terminal, surrounded by a row of cilia larger than those of the general surface. Anal aperture posterior, subterminal.

Coleps hirtus Ehrenberg.

Body barrel-shaped, about twice as long as broad, truncated anteriorly, rounded posteriorly. The mouth is surrounded by tooth-like processes, and there are three spinous processes at the posterior end. Macronucleus spherical, central or subcentral. Contractile vesicle single and posterior. It divides along one of the softer transverse furrows and one half of each new animal lacks, for a time, the armature, as shown in fig. 5, plate I.

This is a very common form found in great abundance in pond water and old infusions, where it appears after most of the other forms of Protozoa have disappeared.

Size varies from 50 to 65 microns. Type specimen, 65 microns. (Figs. 5 and 9, plates XXXIII and XXXIV.)

Genus *Enchelydon* C. & L.

Body ovate or pyriform, elastic, and changeable in form. Anterior end narrower but not prolonged into a neck-like process. Mouth terminal, followed by a longitudinally striated membranous pharynx. Anus posteroterminal.

Enchelydon farctus C. & L.

Body ovate, about twice as long as broad, narrowed anteriorly and usually slightly curved. Pharynx long, narrow and conspicuous. Mouth terminal and projecting slightly beyond the surface of the body. Contractile vesicle single, posterior. Macronucleus band-like, curved. Cilia fine and short.

This species was found in pond water which had been standing in the laboratory for some time.

Size usually given, about 200 microns. Size of type specimen, 180 microns. (Fig. 1, plate XXXIII.)

Genus *Prorodon* Ehr.

Body ovate, cylindrical, rounded at the extremities. Mouth terminal or but slightly subterminal. Pharynx often reënforced by rod-like teeth. Macronucleus spherical or elongated. Contractile vacuole single and posterior. Ciliation complete. A longer tuft of cilia frequently present at the posterior end.

Prorodon teres Ehr.

Body ovate, cylindrical, rounded at the extremities, about twice as long as broad. Mouth terminal. Pharynx provided with rod-like teeth. Macronucleus spherical or elongated. Contractile vacuole single and posterior.

This species is quite common in pond water after long standing.

Length usually given, 150-200 microns. Length of type specimens, 115 microns. (Fig. 3, plate XXXIII.)

Prorodon edentatus C. & L.

Body oval, cylindrical, rounded at the extremities. Mouth eccentrically placed, leading into a simple tube-like pharynx. Macronucleus spherical. Contractile vacuole single and posterior. Cilia at the posterior end slightly longer than those of the general surface. Cuticular surface longitudinally striated.

This species was found but once in pond water after long standing.

Length varies from 120 to 175 microns. Length of type specimens, 120 microns. (Fig. 2, plate XXXIII.)

Prorodon lieberkuhnii Bronn.

The species, represented by fig. 4, plate XXXIII, seems to be identical with one figured by Bronn in *Klassen und Ordnung des Tierreichs* under the name of *Prorodon lieberkuhnii.* It is an elongated form, more than ten times as long as broad. Mouth terminal, elevated above the surface of the body. Pharynx conspicuous, longitudinally striated. Two oval macronuclei located some distance apart. Contractile vacuole single, postero-terminal. Cuticular surface finely striated longitudinally. Cilia relatively long and fine, incoördinate in their movement.

This form was seen but a single time in pond water.

Length of type specimen 300 microns. (Fig. 4, plate XXXIII.)

Genus *Lacrymaria* Ehr.

Body flask-shaped and very contractile, especially in the neck region. Oral aperture terminal, surrounded by one or more rows of cilia. Anal aperture terminal or subterminal. Macronucleus spherical to elongate, sometimes double, centrally located.

Lacrymaria olor Mull.

Body distinctly flask-shaped. Anterior extremity produced into a very long, flexible, neck-like extension. Posterior extremity pointed. Cuticular surface spirally striped. Mouth located in a cone-like projection *Welcher wie ein Pfropf dem Hals der Flasche aufsitzt,* surrounded by a row of long cilia Macronucleus double, centrally located. Two contractile vacuoles.

This is a very beautiful and graceful Ciliate found quite commonly in both pond water and leaf infusions.

Length of type specimen when fully extended, 355 microns; contracted, 100 microns. (Figs. 6 and 8, plate XXXIV.)

Lacrymaria truncata Stokes.

Body elongated flask-shaped. Anterior region narrow and neck-like. Frontal border obliquely truncated. Cuticular surface longitudinally striated. Cantractile vacuole postero-terminal. Macronucleus band-like, very long, coiling back upon itself many times in the posterior region.

Found commonly in hay infusions.

Length of type specimen, 195 microns. (Fig. 10, plate XXXIV.)

Family TRACHELINIDÆ.

Body bilateral, left side flattened, right side convex. Mouth either long and slit-like, extending well down the ventral surface, or round and located some distance from the anterior end. Mouth region usually prolonged into a proboscis. Pharynx short or lacking; when present reinforced by a stiff armature. Ciliation sometimes uniform, sometimes restricted to the flattened surface of the body.

Genus *Lionotus* Wrzesniowskii.

Body elongated, widest centrally and tapering somewhat toward each end. Anterior end more or less pointed, usually flattened and proboscis-like. Posterior end sometimes pointed, sometimes bluntly rounded. Body flexible. Peristome long, extending well down the ventral side of the proboscis to the mouth, which is located at the junction of the proboscis with the body and is usually visible. Contractile vesicle sometimes single, sometimes multiple.

Lionotus wrzesniowskii S. K.

Body elongate lance-shape. Proboscis long, nearly three-fifths the length of entire body, flattened, and slightly dilated at the distal end. Posterior region produced into a pointed tail-like prolongation. Cilia fine and short. An oblique row of trichocysts bordering the entire left margin of the neck. Contractile vesicle single, located at the posterior end of the body proper. Macronucleus double, parts spherical and close together in the center of the body proper. Proboscis and tail-like region hyaline.

Length of type specimen, 155 microns. (Fig. 14, plate XXXV.)

Lionotus fasciola Ehr.

Body lancelate, about five or six times as long as broad, widest centrally and tapering anteriorly into a short, hyaline proboscis which scarcely equals in length one-half of the entire length of body. Tail-like region short and rounded. Under surface ciliated, upper surface naked. Mouth slightly anterior to the middle of the ventral surface. Trichocysts present along the margin of the proboscis. Contractile vesicle single, located in the posterior part of the body. Macronucleus double, parts spherical and close together in the center of the body proper.

Found abundantly in pond water and infusions of leaves and hay.

Sizes given vary from 80 to 120 microns. Size of type specimen, 118 microns. (Fig. 15, plate XXXV.)

Genus *Dileptus* Dujardin.

Body greatly elongated, with a long, narrow, flexible proboscis. Mouth located at the junction of the proboscis with the body proper. Pharynx short but conspicuous. Nucleus variable. Numerous contractile vesicles located along the entire dorsal surface of the body. Trichocysts present in the region of the proboscis.

Dileptus gigas C. & L.

Body greatly elongated. Proboscis varying greatly in length, flexible, but not very extensible. Pharynx short, conical and longitudinally plicate. Macronucleus variable, sometimes ribbon-like. Contractile vesicles very numerous, extending in a dorsal row along practically the entire length of the body. A single ventral row of trichocysts extends along the proboscis. Cuticular surface longitudinally striated.

This species occurs rather commonly in pond water. I have found the greatest possible amount of variation among the individuals of this species. In a single culture I found animals differing widely in size, in relative length of proboscis, and in general shape of body. Some of the individuals were three times as long as others. Some had long proboscides while those of others were short and inconspicuous. In some there was a definite pointed tail-like prolongation, in others the posterior end was bluntly rounded.

I also noticed a difference in the nucleus of the various individuals. Most of the books on classification speak of this species as having a moniliform nucleus; but although I stained a great many of the animals with a variety of stains, I was unable ever to demonstrate such a nucleus. In some of the smaller individuals a band-like nucleus, like the one shown in the figure, was distinctly visible. However, in the great majority of cases the nucleus was plainly scattered. Calkins in his *Protozoölogy* speaks of *Dileptus* as having a scattered nucleus, and this, I believe, is the normal form in the adult animal. (Fig. 11, plate XXXV.)

Genus *Loxodes* Ehr.

Body elongated, leaf-like, flexible, but not persistent in form. Ventral surface flat, longitudinally striated, and finely ciliated. Dorsal surface slightly convex, smooth, and without cilia. Stronger cilia around the margin of the body. Mouth preceded by a ciliated, slit-like peristome. Pharynx tubular, and strengthened by an indurated membrane. Many nuclei. Contractile vacuoles uncertain.

Loxodes rostrum Ehr.

Body flattened, highly flexible, about four times as long as broad. Anterior extremity pointed and curving toward the left. Posterior extremity also pointed and curving slightly toward the left. Mouth on left border of ventral surface at some distance from the anterior end and preceded by a slit-like peristome which runs forward to the apex of the anterior extremity. Walls of pharynx strengthened by a brownish induration. Short marginal setæ present. Nuclei small and numerous. Posterior part of animal very vacuolar. Contractile vacuoles unidentified.

Found in stagnant pond water.

Length varies from 165 to 500 microns. Length of type specimen, 187 microns. (Fig. 12, plate XXXV.)

Genius *Trachelius* Ehr.

Body oval or elliptical. Anterior end produced into a short, dorsally projecting proboscis with round oral aperture at its base. Pharynx short and longitudinally striated. Entire body highly vacular. Cuticular surface finely and uniformly ciliated. Macronucleus central, sometimes single and elongated, sometimes double.

Trachelius ovum Ehr.

Body ovate or nearly spherical, prolonged anteriorly into a short, flexible, snout-like projection. Mouth circular. Pharynx conical, longitudinally striated. Cilia fine and arranged in longitudinal rows. Macronucleus spherical to band-like, sometimes double. Cytoplasm extremely vacuolar. Contractile vacuoles small and numerous.

This species was found but a single time, in water newly brought from a small creek.

Length of type specimen, 295 microns. (Fig. 7, plate XXXIV.)

Family CHLAMYDODONTIDÆ.

Body kidney-shaped or oval. Pharynx distinct, supported by armature of rods or a smooth tube.

Subfamily NASSULINÆ.

Ciliation complete.

Genus *Nassula* Ehr.

Body oval or cylindrical, sometimes slightly flattened, ends broadly rounded. Mouth circular, ventrally located in a slight depression and surrounded by larger and more powerful cilia. The remainder of the body uniformly ciliated and longitudinally striated. Pharynx cylindrical, usually dilated at the exterior, and reinforced by an armature, composed generally of rod-like teeth. Anus terminal. Contractile vesicle, single or multiple. Body usually brightly colored. Macronucleus spherical to bandlike. Trichocysts present or wanting.

Nassula aurea Ehr.

Body elongated, cylindrical. Pharynx armed with rod-like teeth and dilated at the exterior. Macronucleus spherical and located posterior to the center of the body. Trichocysts wanting. A transverge groove present dorsal to mouth region. Contractile vesicle single. Body usually brightly colored with green and reddish brown.

This species was found twice in water newly taken from a small creek.

Length of type specimen, 216 microns. (Fig. 17, plate XXXVI.)

Nassula rubens C. & L.

Body cylindrical, elongated, ends equally rounded. Pharynx conspicuous with rod-like teeth, dilated at exterior. Trichocysts abundant. Macronucleus spherical, subcentral. Contractile vacuole single. Body usually reddish-brown in color.

This species was found but once, in water newly taken from a small creek.

Length of type specimen, 110 microns.

Subfamily CHILODONTINÆ.

Body usually flattened ventrally, convex dorsally. Ciliation either confined to the ventral side or much stronger than on dorsal side.

Genus *Chilodon* Ehr.

Body compressed, with a flattened ventral surface which is striated and bears cilia. Soft, flexible anterior end bends to the left in a lip-like projection. Posterior end usually rounded. Mouth median or nearly so. Pharynx conical, provided with from ten to sixteen rods. Macronucleus oval, centrally located; one micronucleus. Contractile vesicles varying from one to many.

Chilodon cucullus Mull.

Body elongated oval, about twice as long as broad, rounded posteriorly. Lip-like extension prominent. A groove and a line of stronger cilia leading from the end of this projection to the mouth. Contractile vesicles scattered, varying in number with the size of the individual. Macronucleus single, situated posterior to the inner end of the pharynx.

This is a very common species, found usually in pond water.

Sizes given vary from 100 to 398 microns. Length of type specimen, 117 microns. (Fig. 13, plate XXXV.)

Chilodon megalotrocha Stokes.

The form illustrated by fig. 16, plate XXXV, seems to be identical with *Chilodon megalotrocha* as figured by Conn in his *The Protozoa of the Fresh Waters of Connecticut.* It is a very small, clear animal, corresponding to the generic description. Pharynx supported by minute rods, siuated anterior to the middle of the ventral surface. Macronucleus single, subcentral. Usually two contractile vacuoles.

A very common species, found in both pond water and hay infusions.

Length of type specimen, 48 microns. (Fig. 16, plate XXXV.)

Suborder TRICHOSTOMINA.

Mouth permanently open. Undulating membranes usually present in mouth region.

Family CHILIFERIDÆ.

Mouth never posterior to middle of the body. Undulating membrane usually present in connection with mouth. Pharynx short when present, often lacking. Peristomal area either entirely lacking or but faintly developed.

Genus *Frontonia* Ehr.

Body elongated oval, cylindrical or slightly flattened dorsoventrally. Extremities either rounded or pointed. Mouth permanently open, located anterior to the middle of the body. Left-hand border of the mouth with undulating membrane; right-hand border with elevated ridges. A groove extends from the mouth posteriorly, often to the extreme end of the body. Pharynx short and inconspicuous. Cuticular surface longitudinally striated with rows of fine cilia. Trichocysts abundant, lacking in mouth region. One or two contractile vacuoles. Macronucleus oval; one to many micronuclei.

Frontonia leucas Ehr.

Body elongated oval, anterior extremity widest. Rounded anteriorly, pointed posteriorly. Mouth anterior to the middle of the body, an undulating membrane on its left-hand border; right-hand border with ridges; a groove extending from the mouth well down the body. Cilia very fine. Trichocysts abundant.

This species was found frequently in pond water.

Average length given, 275 microns. Length of type specimen, 240 microns. (Fig. 22, plate XXXVII.)

Genus *Colpidium* Stein.

Body oval or reniform, incurved ventrally, arched dorsally. Posterior extremity somewhat broader. Mouth located in a depression some distance from the anterior end. Pharynx tubular with projecting undulating membrane. Anus terminal. Contractile vacuole terminal or forward on the dorsal side. Macronucleus spherical; micronucleus single, *Colpidium striatum* Stokes.

Body elongated reniform, at least twice as long as broad. Anterior extremity curved slightly toward the ventral side. Macronucleus spherical, centrally located. Contractile vacuole near posterior extremity.

Found in abundance in vegetable infusions.

Length of type specimen, 110 microns. (Fig. 24, plate XXXVII.)

Colpidium putrinium Stokes.

Body ovate, less than twice as long as broad. Anterior end obtusely pointed. Ventral surface flattened slightly. Macronucleus spherical. Undulating membrane small. Contractile vacuole single. Cuticular surface longitudinally striated.

Length of type specimen, 56 microns. (Fig. 23, plate XXXVII.)

Genus *Colpoda* Mull.

Body persistent in form, oval or reniform, compressed laterally, with convex dorsal surface. Oral aperture in a deep depression a short distance from the anterior extremity. No undulating membrane, but a few stronger cilla located at the posterior border of the mouth. Pharynx absent or inconspicuous. Surface longitudinally striated. Cilia very fine. Resembles *Colpidium* very closely, but differs in the motor apparatus connected with the mouth.

Colpoda cucullus Ehr.

Body reniform, about one and a half times as long as broad. Posterior end broad and bluntly rounded; anterior end narrower and less bluntly rounded. Body curved toward the ventral side. Striations conspicuous only at the extremities, and especially at the anterior extremity. Macronucleus spherical, almost central in position. A single micronucleus. Contractile vacuole single, posteriorly located.

This is a very common form in infusions of hay and leaves.

Length of type specimen, 95 microns.

Colpoda helia Stokes.

Body elongated reniform, rounded at both extremities. Anterior extremity slightly curved toward the ventral aspect. Mouth situated about one-third the length of the body from the anterior end. Pharynx short. Macronucleus ovoid, nearly central in position. Contractile vesicle in the posterior half of the body.

Length of type specimen, 175 microns. (Fig. 21, plate XXXVII.)

Genus *Tillina* Gruber.

Body bean-shaped, persistent in form. Mouth located near the middle of the ventral side. Pharynx long and recurved. Cuticular surface clothed with fine cilia. Longer cilia bordering the mouth and continued down the pharynx. Macronucleus large, oval. Contractile vesicle single, postero-terminal.

I have taken my classification of this genus from Miss Louise Hoyt Gregory, who, in her article on *The Life History of Tillina magna*, places it in the family Chiliferidæ.

Tillina magna Gruber.

Body broadly bean-shaped, laterally compressed, about twice as long as broad. Contractile vacuole located in a lobe which extends dorsally from the posterior part of the body. Oral aperture near the middle of the ventral surface. Pharynx recurved and dilated at its inner end, its walls completely ciliated. Cuticular surface smooth. Apparent striations radiating from the posterior part of the animal forward, in the lower layer of the body. Macronucleus oval, located in the anterior half of the body.

Although this is generally considered to be a rare species, it appeared in two of my hay infusions in great abundance, and lived for several weeks without any special care. This was in the early spring. Reproduction occurred frequently through the formation of cysts and subsequent division into two or four. Miss Gregory found the species in a culture of horse manure, and regarded it as a possible parasite of the horse. I have found nothing to indicate that this is so.

Length of type specimen, 210 microns. (Fig. 26, plate XXXVIII.)

Family PARAMÆCIDÆ.

Mouth in either the anterior or the posterior half of the body, preceded by an oblique, somewhat triangular furrow.

Genus *Paramæcium* Mull.

Body oval or elongate, persistent in form but quite flexible. Cuticular surface uniformly ciliated. An oblique, triangular buccal groove leads to the mouth, which is near the center of the ventral side. Contractile vesicles usually two. Macronucleus large, oval or ellipsoidal; two or more micronuclei. Trichocysts abundant.

Paramæcium caudatum Ehr.

Body elongated, somewhat cigar-shaped, with the posterior half slightly inflated. Rounded at the anterior extremity and pointed posteriorly. A triangular buccal groove extends obliquely backward from the anterior extremity to beyond the middle of the body. Mouth located at the posterior end of the buccal grove. Pharynx short, curved, and ciliated. Macronucleus large, centrally located; micronucleus single. Contractile vesicles two, located at about one-third the length of the body from each extremity. Trichocysts abundant and long.

This is perhaps the most common and most widely known Ciliate, and it can be found in either pond water or vegetable infusions. It varies greatly in size.

Length of type specimen, 285 microns. (Fig. 25, plate XXXVIII.)

Paramæcium bursaria Ehr.

Body slightly elongated, about twice as long as broad. Obliquely truncated anteriorly, broadly rounded posteriorly. Buccal groove triangular, very wide, extending from the anterior end to beyond the middle of the body. Anus postero-terminal. Two contractile vesicles, one some distance from either end. Macronucleus large, centrally located; micronucleus single. Trichocysts abundant and strongly developed. Cytoplasm usually greenish.

This is apparently a somewhat rare species. Found in pond water.

Sizes given range from 90 to 122 microns. Length of type specimen, 122 microns. (Fig. 28, plate XXXVIII.)

Family PLEURONEMIDÆ.

Body compressed dorsoventrally or laterally. A long peristome on the ventral side leads to the mouth. Peristomal area provided with one or more undulating membranes.

Genus *Cyclidium* Ehr.

Body persistent in form, oval, compressed dorsoventrally. Oral aperture ventral with extensible hood-like membrane. Cuticular surface with fine, long, rigid cilia. One or more long caudal setæ on the posterior border. Contractile vesicle single, posteriorly located. Macronucleus spherical.

Cyclidium glaucoma Ehr.

Body ovate, slightly concave ventrally and convex dorsally, about twice as long as broad. Mouth slightly anterior to the middle of the body, supplemented by a hood-like membrane. Cilia very long and slender, arranged in longitudinal rows. Caudal setæ single, twice the length of the other cilia. Macronucleus spherical, nearly central in position. Contractile vacuole single.

This is an extremely common form, found both in pond water and in infusions. The fact that the membrane is very difficult to detect makes it liable to be placed in the wrong genus.

Length, 20 microns. (Fig. 27, plate XXXVIII.)

Order HETEROTRICHA.

Ciliata which are entirely ciliated but with an adoral zone of specialized cilia of great size or fused together into membranelles. These specialized cilia constitute a circular or spiral peristomal series.

Suborder POLYTRICHINA.

Heterotricha with a uniform coating of cilia.

Family PLAGIOTOMIDÆ.

Peristomal area a long, narrow furrow, usually extending from the anterior extremity to the mouth. Mouth generally posterior to the middle of the body. Adoral zone extending along the left-hand edge of the peristome.

Genus *Nyctotherus* Leidy.

Body ovate or reniform, somewhat compressed. Peristome cleft-like, extending on the ventral side from near the anterior extremity to the middle of the body, then turning backward and inward to the pharynx. Adoral cilia on the left-hand border of the peristome. Pharynx with long cilia. Anus permanent, connected with a fissure-like rectal passage. Contractile vacuole single, subcentral. Macronucleus ovate, anterior to the pharynx. Parasites in amphibians and invertebrates.

Nyctotherus cordiformis Stein.

Body ovate to reniform, compressed, about two-thirds to three-fourths as broad as long. Anterior end slightly pointed. Entrance to pharynx bearing long, stiff setæ on its lower edge. Pharynx conspicuous, long and recurved. Cuticular surface with longitudinal striations. Macronucleus elongated oval; micronucleus single. Contractile vacuole postero-terminal.

This is a common parasite in the intestinal tract of frogs.

Length of type specimen, 175 microns. (Fig. 32, plate XXXIX.)

Genus *Metopus* C. & L.

Body elongated oval, cylindrical, highly elastic and changeable in form. Anterior extremity usually twisted obliquely upward, overlapping the ventral surface. Peristome a long, narrow furrow, extending obliquely from left to right. Pharynx short. Mouth near or posterior to the median line. Body striated and ciliated, longer cilia at the anterior and posterior extremities. Macronucleus cylindrical. Contractile vacuole postero-terminal.

Metopus sigmoides Mull.

Body normally elongate, quite changeable in form, about three times as long as broad. Anterior extremity rounded, posterior extremity bluntly pointed. Body striated and covered with cilia, an especially long tuft at the posterior end. Macronucleus oval, centrally located; micronucleus single. Contractile vacuole large, terminal.

Found in pond water.

Sizes given range from 83 to 260 microns. Size of type specimen, 210 microns. (Fig. 29, plate XXXIX.)

Genus *Metopides* Quennerstedt.

Body persistent in form, ovate or pear-shaped. Anterior region obliquely twisted toward the left over the ventral surface. The groove formed thus is the oral groove and leads to the mouth. Pharynx short. Oral groove strongly ciliated. Cuticular surface entirely covered with fine cilia. Tuft of setæ at the posterior end.

Metopides acuminata Stokes.

Body pear-shaped. Anterior extremity broad and rounded; tapering posteriorly to a tail-like region. Several long, slender setæ at the posterior end. Macronucleus spherical, centrally located. Contractile vacuole single, posterior.

Length of type specimens, 73 microns. (Fig. 31, plate XXXIX.)

Genus *Spirostomum* Ehr.

Body cylindrical or worm-like, greatly elongated, usually rounded anteriorly and truncated posteriorly. Oral groove extending from the anterior extremity down the ventral surface to the middle of the body or beyond. Adoral cilia bordering the left-hand side of the peristome. Cuticular striated spirally. Macronucleus ovate or moniliform. Anus and contractile vacuole terminal. A tubular canal extending from the contractile vacuole to the anterior part of the body.

Spirostomum ambiguum Ehr.

Body from ten to sixteen times as long as broad. Peristome extending to or beyond the middle of the body. Macronucleus moniliform. Contractile vacuole often occupying a third or more of the body, with the canal-like region extending almost to the anterior end.

This is the longest species of Ciliate which I have yet seen. It is quite conspicuous to the unaided eye.

Found abundantly in pond and creek water.

Lengths given vary from 520 to 4000 microns. Length of type specimen, 1450 microns. (Fig. 35, plate XL.)

Spirostomum teres C. & L.

Body not so long in proportion to width as the preceding species, narrower and rounded anteriorly, truncated posteriorly. Peristome not reaching the middle of the body. Macronucleus single, ovate.

This species is found wherever *Spirostomum ambigumm* is found. It is considerably smaller than that species.

Sizes given range from 300 to 650 microns. Length of type specimen, 650 microns. (Fig. 33, plate XL.)

Family BURSARIDÆ.

Peristomal area broadly triangular and deeply sunken.

Genus *Condylostoma* Duj.

Body ovate to elongate, nearly cylindrical. Peristome short, triangular. Left-hand border of peristome with adoral cilia, right-hand border with undulating membrane. Mouth large, located at the apex of the peristomal triangle. Pharynx small. Cuticular surface finely ciliated. Macronucleus bead-like. Contractile vacuole varying. Anus postero-terminal.

Condylostoma stagnale Wrz.

Body broadly ovate, less than twice as long as broad, widest posteriorly. Peristomal area broadly triangular, occupying the entire anterior end and extending posteriorly to about the middle of the body. Left-hand border of the peristome with adoral cilia, right-hand border with a large undulating membrane. Longitudinally striated with conspicuous muscular fibrillæ. Macronucleus bead-like. Contractile vacuole irregular in outline, postero-terminal.

This species was observed but once, in stagnant pond water.

Average size, 200 microns. Length of type specimen, 150 microns. (Fig. 30, plate XXXIX.)

Family STENTORIDÆ.

Peristome limited to the surface of the anterior end of the animal, at right angles to the long axis of the body. Undulating membranes lacking. Peristomal area straited with rows of strong cilia.

Genus *Stentor* Oken.

Protozoa either free-swimming or attached at will, sometimes secreting a mucilaginous case or lorica. Pyriform, pear-shaped or ovate when unattached; trumpet-shaped when attached and expanded. Peristome circular, a spiral row of cilia around it with the left-hand end of the spiral depressed and leading into the mouth. Pharynx short. Cuticular surface longitudinally striated and covered with fine cilia. Long slender setæ sometimes present on the general surface. Macronucleus moniliform, band-like or oval; micronuclei numerous. Anus and contractile near left-hand side of the adoral wreath. Contractile vacuole, usually with tube-like canal, leading from the posterior part of the body.

Stentor roeselii Ehr.

Body large, soft, and transparent; highly elastic. When fully expanded, anterior end rarely measures one-fourth the total length. Sometimes secreting a transparent mucilaginous sheath. Cuticular surface striated and finely ciliated, often bearing slender setæ which project rigidly at right angles to the body. Fine, short setæ developed on adhesive posterior end. Macronucleus elongated, usually band-like.

Sizes range from 500 to 1044 microns. Length of type specimen expanded 675 microns.

Found in stagnant pond water.

The species here pictured agrees entirely with *Stentor roeselii*, but has not been found inhabiting a mucilaginous sheath. (Fig. 34, plate XL.)

Stentor cæruleus Ehr.

Body very large and bulky. Color more or less intensely blue. Muscular striæ conspicuous. Cilia of the general surface fine, with occasional long, slender setæ, which are not conspicuous. Macronucleus moniliform.

This species was found in abundance in stagnant pond water; social. It seems quite evidently to be an extremely large variety of *Stentor cæruleus*.

Lengths generally given range from 130 to 300 microns. Length of type specimen unattached, 875 micons. (Figs. 37 and 38, plate XLI.)

Stentor polymorphus, Mull, which differ from *Stentor cæruleus* chiefly in being intensely green, was observed but not drawn.

Suborder OLIGOTRICHINA.

Heterotricha having the cilia limited to certain areas.

Family HALTERIIDÆ.

Cilia limited to an adoral wreath and sometimes a posterior circle of springing hairs.

Genus *Halteria* Duj.

Body nearly spherical. Mouth eccentric. Adoral wreath of large cilia spiral or subcircular. A zone of long setæ or springing-hairs developed in the equatorial region. Body otherwise unciliated.

Halteria grandinella Mull.

Body nearly spherical, rounded posteriorly. Adoral wreath forming an involution which leads to the mouth. Springing-hairs long, not forming an equatorial girdle. Macronucleus spherical or oval. Contractile vacuole single.

A very common species, found in pond water and vegetable infusions. Size ranges from 16 to 50 microns. (Fig. 36, plate XLI.)

Order HYPOTRICHA.

Ciliata having the cilia limited to the ventral surface. Body usually flattened dorsoventrally.

Family OXYTRICHIDÆ.

Peristome somewhat indistinct. Cilia limited to a few on the ventral surface, usually arranged in rows or groups. Anal and frontal styles present.

Genus *Urostyla* Ehr.

Body flexible, elongated oval, flattened ventrally, convex dorsally. Peristome acutely triangular. Three or more frontal styles; several rows of ventral styles; from 5 to 12 anal styles, set in an oblique row; an uninterrupted border of marginal setæ. Macronucleus single or multiple. Contractile vacuole single, located near the end of the peristome on the left-hand border.

Urostyla grandis Ehr.

Body ovate, variable in form, from three to four times as long as broad. Peristome field narrowly triangular, about one-third the length of the body, its reflected border ciliated. Numerous frontal styles between the reflected border of the peristome and the right-hand margin of the body; several slender anal styles, obliquely set; numerous rows of ventral cilia. Usually two macronuclei, each with its attached micronucleus.

Found in infusions of hay and leaves.

Lengths given vary from 250 to 410 microns. Length of type specimen, 250 microns. (Fig. 48, plate XLIV.)

Genus *Uroleptus* Ehr.

Body narrow, greatly elongated, persistent in form but highly flexible. Anterior extremity rounded, posterior extremity prolonged into a tail-like projection. Three or four frontal styles; usually two median longitudinal rows of ventral setæ; no anal styles; marginal setæ well set in on the ventral surface. Contractile vacuole located near the posterior end of the peristome.

Uroleptus agilis Ehr.

Body greatly elongated, four to five times as long as broad, widest centrally, rounded anteriorly, prolonged posteriorly into a narrow, tail-like prolongation, which sometimes curves toward the right. Peristome narrowly triangular, about one-fifth the length of the body. Four frontal styles; two median rows of ventral styles, additional ventral styles sometimes produced; marginal series of setæ complete, somewhat longer in the posterior region. Macronuclei ovate, two in number. Contractile vacuole located anterior to the middle of the left-hand border.

Length given varies from 83 to 250 microns. Length of type specimen, 180 microns. (Fig. 40, plate XLII.)

Genus *Onychodromus* Stein.

Body nearly rectangular, extremities more or less rounded. Peristome broadly triangular, extending to the middle of the body. Three rows of frontal styles, parallel to the peristome and located between its reflected border and the right-hand margin of the body; three or four rows of ventral styles; an uninterrupted series of marginal setæ; five or six anal styles. Usually four macronuclei with attached micronuclei. Contractile vacuole single, located on the left-hand side, near the posterior angle of the peristome.

Onychodromus grandis Stein.

Body somewhat rectangular, about two and a half times as long as broad. Lateral margins nearly parallel; anterior margin obliquely truncated on the left side in adult animals. Peristome field broadly triangular, its reflected border with an undulating membrane. Three large anterior styles, followed by three rows of smaller frontal styles; numerous ventral styles in three or four rows; from four to seven anal styles. Four ovate macronuclei, each with a small micronucleus.

This species was found several times in pond water. At one time a peculiar modification was present. Each of the marginal setæ was fibrillate or branched. This feature occurred in each individual examined from the one culture, but had not been noticed in the earlier cultures. It was probably a local modification. I have noticed that under certain conditions the styles of many Hypotricha, normally unbranched, become branched irregularly.

Lengths range from 104 to 347 microns. Length of type specimen, 255 microns. (Fig. 39, plate XLII.)

Genus *Gastrostyla* Engelmann.

Body persistent in form, elongated oval. Peristome triangular, its reflected border bearing an undulating membrane. Five or six frontal styles; several ventral styles arranged in an oblique row; five or six anal styles; an uninterrupted series of marginal setæ, the most posterior usually being the longest. Two or four macronuclei.

Gastrostyla steinii Engelmann.

Body elongated oval, narrowest anteriorly, rounded at the extremities. Between two and three times as long as broad. Peristome triangular, about one-third the length of the body. Six long frontal styles; an oblique row and two or three additional ventral styles; four or five anal styles, not projecting beyond the posterior margin of the body. Marginal setæ longest at the posterior extremity. Four ovate macronuclei. Contractile vacuole single, located at the left side near the posterior angle of the peristome.

Length varies from 150 to 330 microns. Length of type specimen, 156 microns. (Fig. 41, plate XLII.)

Genus *Oxytricha* Ehr.

Body rather flexible, elongated oval. Three to several frontal styles; a few scattered ventral styles; five anal styles; an uninterrupted series of marginal setæ. Two to four macronuclei. Contractile vacuole single.

Oxytricha bifaria Stokes.

Body oval, less than three times as long as broad, narrowest anteriorly. Right border convex, left border concave or flattened. Posterior end bluntly pointed. Seven frontal styles, five scattered ventral styles, and five anal styles. Peristome reaching to the middle of the body, right border ciliated and bearing a narrow membrane. Marginal setæ uninterrupted, stronger at the posterior end. Macronucleus usually double. Contractile vacuole anterior to the middle of the body.

This is described as being a quite variable species. Average length, 250 microns. Length of type specimen, 200 microns. (Fig. 45, plate XLIII.)

Oxytricha pellionella Mull.

Body elastic but persistent in form, more than four times as long as broad, slightly widest centrally and tapering to each end. Extremities equally rounded. Peristome narrowly triangular, relatively short. Three large hooked frontal styles, and three small straight ones; five scattered ventral styles; five long anal styles, projecting beyond the posterior margin of the body; an uninterrupted series of marginal setæ. Two macronuclei and two micronuclei. Contractile vacuole located near the middle of the left-hand border.

Found in an infusion of hay and leaves.

Sizes range from 86 to 160 microns. Length of type specimen, 160 microns. (Fig. 46, plate XLIII.)

Genus *Stylonychia* Ehr.

Body persistent in form, inflexible, elongated or elliptical. Dorsal surface convex, ventral surface flat. Eight frontal, five ventral, and five anal styles; three long, hair-like caudal setæ. Marginal setæ forming a continuous border on each side of the body. Two macronuclei. Contractile vacuole single, located on the left-hand side of the body, near the posterior angle of the peristome.

Stylonychia mytilus Ehr.

Body elongated oval, more than twice as long as broad, widest anteriorly and tapering to the posterior end. Posterior third of the body often quite narrow and curved. Both extremities rounded. Peristome large, broadly triangular, its reflected border with an undulating membrane. The two right-hand anal styles projecting beyond the posterior extremity of the body; caudal setæ long, slender, and widely separated, interrupting the marginal series.

Found in pond water.

Sizes given vary from 90 to 400 microns. Length of type specimen, 160 microns. (Fig. 43, plate XLIII.)

Stylonychia pustulata Ehr.

Body elongated oval, about twice as long as broad, rounded at the extremities, not tapering. Caudal setæ short, close together, scarcely interrupting the marginal series; most of the anal styles projecting beyond the posterior border. Two macronuclei and two micronuclei.

Found in vegetable infusions.

Lengths given vary from 90 to 170 microns. Length of type specimen, 105 microns. (Fig. 42, plate XLIII.)

Stylonychia notrophora Stokes.

Body elongated, elliptical. Left side of anterior margin obliquely truncated; posterior extremity rounded. Peristome broadly triangular, extending to near the middle of the body. Two or three of the anal styles project beyond the margin of the body; caudal setæ long, slender and widely separated. Two macronuclei and two micronuclei.

Length of type specimen, 115 microns. (Fig. 44, plate XLIII.)

Family EUPLOTIDÆ.

Body usually oval. Cilia greatly reduced. Frontal, ventral, and marginal cirri reduced in number; marginal cirri often wanting. Nucleus spherical to band-like.

Genus *Euplotes* (Ehr.) Stein.

Body inflexible, persistent in form, oval in outline; flattened ventrally, convex dorsally. Both ends usually rounded; anterior end occasionally truncated. Peristome reaching to or beyond the middle of the body. Six to eight frontal styles; a few scattered ventral styles; five anal styles; usually four marginal setæ, two at the posterior end and two on the posterior part of the left-hand border. Dorsal surface usually with ridges. Contractile vacuole near the insertion of the anal styles. Anus posterior and to the right. Macromnucleus long, band-like.

Euplotes patella Ehr.

Body elongated oval, usually somewhat truncated anteriorly, rounded posteriorly. Peristome wide, extending to or beyond the center of the body, its reflected border a simple ciliated groove. Six frontal, three scattered ventral, and five anal styles; four marginal setæ, the two posterior often branched. Contractile vacuole beneath the insertion of the two right-hand anal styles. Macronucleus long, bandlike and curved.

Found in infusions of grass, hay and leaves.

Sizes given range from 125 to 155 microns. Length of type specimen, 155 microns. (Fig. 47, plate XLIV.)

Euplotes carinata Stokes.

Body oval, rounded at the extremities, left border obliquely truncate in two directions, producing an angle. Peristome extending to about the middle of the body. Dorsal surface usually grooved. Seven frontal, three ventral, and five annal styles; four marginal setæ. Macronucleus bandlike, curved. Contractile vacuole single, located beneath the insertion of the anal styles.

This form has been found in abundance in infusions of leaves.

Length of type specimen 95 microns. This is probably an abnormally large individual. (Fig. 49, plate XLIV.)

Genus *Aspidisca* Ehr.

Body small, inflexible, persistent in form, oval in outline, convex dorsally, flattened ventrally. Left side of body nearly straight, right side convex. Right-hand margin of ventral surface thickened. Peristome extending to beyond the middle of the body. Left margin of the peristome often forming a cover for the anterior end. Seven or eight styles on the anterior portion of the ventral surface; five to twelve on the posterior portion; marginal setæ lacking. Anus and contractile vacuole near the insertion of the anal styles.

Aspidisca costata Duj.

Body rounded oval, dorsal surface convex, ventral surface slightly flattened. Five or six longitudinal grooves present. Peristome with lip-like extension or cover. Three frontal styles; four or five scattered ventral styles; five anal styles.

This small species is very common in pond water. An accurate drawing is difficult to get because of its small size and the fact that it persistently climbs around and around some small particle of debris. When not thus supported, as Dr. Edmondson points out, it tumbles about in a very clumsy fashion.

Average length, 35 microns. (Fig. 50, plate XLIV.)

Order PERITRICHA.

Ciliata in which the cilia have been greatly reduced. Cilia usually restricted to the adoral zone but occasionally being present in the form of secondary wreaths.

Family VORTICELLIDÆ.

Body often cup- or bell-shaped. Celia reduced to a dexiotropic, spiral adoral zone, and a secondary posterior wreath which may be present or wanting, permanent or temporary. Forms attached or unattached.

Subfamily URCEOLARINÆ.

Forms having a permanent secondary wreath of the cilia and an inclosed adhesive disc, but no contractile peristomal fold.

Genus *Trichodina* Ehr.

Forms parasitic, usually solitary and unattached. Body discoidal, conical, or cylinderical. Mouth eccentric. Adoral wreath spiral, leading into a short pharynx. Posterior extremity with a fringe of long cilia, and reinforced by a horny ring and denticles. Macronucleus moniliform or band-like. Contractile vacuole single, near the inner end of the pharynx.

Trichodina sp.

This animal was found within the shell of a small living snail (*Physa* —). I have been unable to classify it as to species, though it agrees in many respects with *Trichodina pediculus* Ehr. Body discoidal, never hourglass shape, equal in length to about two-thirds diameter. Cilia of the posterior end longer than those of the adoral wreath. Horny ring of the posterior region supplemented by an outer and an inner series of denticles. Macronucleus moniliform.

Width of type specimen, 80 microns; length, 50 microns. (Figs. 66 and 67, plate XLVI.)

Family GYROCORIDÆ Stein.

Free-swimming Peritricha, persistent in form. Body ovate or pyriform, with one or more ciliary wreaths. Oral aperture lateral or ventral. Anal aperture postero-terminal.

Genus *Telotrochidium* S. K.

Body oval or campanulate. Ciliary circles two. No caudal appendages. Mouth ventral, behind the anterior wreath of cilia. Anal aperture postero-terminal.

Telotrochidium sp.

Fig. 64 represents a species which undoubtedly belongs to this genus, though no postero-terminal anal aperture was observed. Body oval, narrowest at the anterior end. Anterior border thickened. Pharynx long and conspicuous. Macronucleus band-like. Two contractile vacuoles.

This is a very common species in infusions of hay and leaves.

Length of type specimen, 95 microns. (Fig. 64, plate XLVI.)

Subfamily VORTICELLINÆ.

Forms usually cup- or bell-shaped, without a permanent secondary wreath of cilia. Contractile peristomal fold present, enabling the animal to enclose the peristome.

Genus *Scyphidia* Lachman.

Forms solitary, unattached. Body elongated, pyriform, highly contractile. Posterior end with an organ of attachment. Surface smooth or striated. Mouth anterior, eccentric. Pharynx distinct. An elevated ciliary disc, surrounded by a fringe or adoral cilia, descends into the oral aperture.

Scyphidia inclinans D'Udk.

Body highly contractile, somewhat pyriform, slightly widest centrally, more than twice as long as broad. Posterior end narrow and stalk-like. Ciliary disc elevated obliquely. Pharynx conspicuous, extending to the middle of the body. Cuticular surface smooth. Macronucleus band-like. Contractile vacuole single, located near the pharynx and just beneath the peristome border. Body slightly bent to one side when not fully extended.

Found in pond water among algæ.

Length of type specimen, not fully extended, 60 microns. (Fig. 63, plate XLVI.)

Genus *Vorticella* Linnaeus.

Body usually cup- or bell-shaped, attached posteriorly to an unbranched, contractile stalk. Adoral mechanism consisting of an elevated ciliary disc, encircled by an adoral wreath of large, strong cilia which descends to the mouth. Mouth eccentric. Pharynx usually conspicuous, tube-like. Contractile vacuole single or double, located near the pharynx. Macronucleus band-like, curved. Cuticular surface either smooth or transversely striated. Individuals often social, never colonial.

The classification of the various species of *Vorticella* is very difficult because of the slight amount of variation between species, the ease with which the individuals vary in contour, and the great number of species based upon minor differences. There is very little to build upon in classification. Calkins believes that the 66 species enumerated by Stokes and the several new species added since his work might safely be reduced to 12 or 15 species, and this is perhaps true. I do not, therefore, expect every one to agree with me in my classification of *Vorticella*.

Vorticella gracilis Duj.

Body conical, about twice as long as broad. Anterior margin slightly everted. Contractile vacuole single. Cuticular surface smooth. Body transparent. Stalk slender, about one and a half times the length of the body. Length of type specimen 70 microns. (Fig. 53, plate XLV.)

Vorticella cucullus From.

Body slender, conical, about three times as long as the width, tapering posteriorly. Anterior border slightly everted. Ciliary disc cushion-like. Contractile vacuole single. Cuticular surface smooth. Body transparent. Stalk about the length of the body, slender.

Length of type specimen, 85 microns. (Fig. 51, plate XLV.)

Vorticella longifilum S. K.

Solitary. Body slender, about twice as long as broad. Peristome somewhat dilated. Cuticular surface smooth. Stalk slender, from ten to twelve times the length of the body.

Length of type specimen, 53 microns. (Fig. 52, plate XLV.)

Vorticella campanula Ehr.

Body quite variable in form, broadly bell-shaped. Anterior margin dilated and slightly everted, sometimes exceeding in width the length of the body. Stalk thick, three to seven times the length of the body. Cuticular surface smooth. Eminently social.

Length of type specimen, 85 microns. (Fig. 59, plate XLV.)

Vorticella microstoma Ehr.

Body variable in form, usually ovate or nearly globular, a little longer than broad. Border of peristome narrow and constricted, about one-half the width of the center of the body. Cuticular surface finely striated. Pharynx long. Contractile vacuole single. Stalk slender, from two to six times the length of the body. Solitary.

Length of type specimen, 55 microns. (Fig. 54, plate XLV.)

Vorticella utriculus Stokes.

Body subpyriform, about twice as long as broad. Width of peristome about two-thirds that of the center of the body. Body slightly constricted beneath the peristome border. Peristome border everted. Cuticular surface strongly striated transversely. Pedicle two to four times the length of the body.

Lengths given range from 33 to 40 microns.

Length of type specimen, 38 microns. (Fig. 61, plate XLVI.)

Vorticella elongata From.

Body conical, elongated, between two and three times as long as broad. Posterior extremity tapering to a conical point. Anterior end slightly dilated, with raised peristome border. Ciliary disk somewhat elevated. Cuticular surface transversely striated. Stalk stout, about two and a half times the length of the body.

Length of type specimen, 95 microns. (Fig. 60, plate XLVI.)

Vorticella spectabilis S. K.

Body quite variable in form, conical, elongated, between two and three times as long as broad. Anterior end expanded, peristome everted. Cuticular surface finely straited transversely. Stalk short, from one to three times the length of the body. Contractile vacuole single. Social.

This species was found in abundance in pond water in large social clusters.

Length of type specimen, 95 microns. (Fig. 62, plate XLVI.)

Vorticella convallaria Linn.

Body elongated, bell-shaped, nearly twice as long as broad. Anterior margin slightly dilated, everted. Cuticular surface transversely striated. Stalk short and thick, four to six times the length of the body. Contractile vacuole single.

Length of type specimen, 130 microns. (Fig. 55, plate XLV.)

Vorticella pusilla Stokes.

Body minute, conical, less than twice as long as broad. Width of peristome about equal to the center of the body. Body constricted beneath the peristome, widest behind this constriction and tapering thence to the posterior end. Stalk four to six times the length of the body.

Length of type specimens, 18 microns. (Fig. 58, plate XLV.)

Vorticella aquæ-dulcis Stokes.

Body ovate to pyriform, a little longer than broad. Width of peristome a little greater than one-half the width of the center of the body, border thickened. Ciliary disc obliquely elevated. Pedicle two or four times the length of the body.

Length of type specimen, 35 microns. (Fig. 56, plate XLV.)

Vorticella similis Stokes.

Body broadly campanulate, nearly as wide as long, changeable in shape. Body tapering gradually from anterior to posterior end, constricted beneath the border of the peristome. Peristome equal in width to the length of the body, its border revolute. Pedicle three to seven times the length of the body. Cuticular surface transversely striated.

Length of type specimens, 70 microns. (Fig. 57, plate XLV.)

Genus *Charchesium* Ehr.

Zoöids similar to *Vorticella* but forming branched colonies; individuals of a colony not differing in size and shape. Central muscle fibre of common pedicle not continuous in the branches but interrupted at the base of each individual stalk, permitting the zooids to contract independently.

Carchesium polypinum Linn.

Bodies somewhat bell-shaped, peristomal border dilated. Cuticular surface smooth. Pharynx long, curved. Macronucleus bandlike, curved. Contractile vacuole single, located near the pharynx. Common pedicle subdivided. Pedicles of the individual zoöids usually springing from the subdivisions.

This species was found in abundance in stagnant pond water. Many of the colonies are large and beautiful.

Length of zooids from 50 to 75 microns. (Figs. 69 to 70, plate XLVII.)

Genus *Epistylis* Ehr.

Zooids similar to *Vorticella,* but forming colonies which are usually dichotomously branched. Individuals usually similar in size and shape. Pedicle treelike, stout, and rigid, with no contractile elements.

Epistylis plicatilis Ehr.

Bodies conical, elongated, about three times as long as broad. Anterior margin expanded. Ciliary disc considerably elevated. Cuticular surface smooth. Posterior end conically pointed, forming annular folds when contracted. Macronucleus short, oval to bandlike. Contractile vacuole single. Pedicle slender, dichotomously branched; secondary branches long; unbranched portion short. Pedicle longitudinally striated.

This species was found attached to sticks and weeds in fresh pond water. The suctorian, *Podophrya quadripartita*, was often found attached to the branches of this species.

Lengths given for single zooids vary from 75 to 175 microns. Length of type specimen, 85 microns. (Figs. 71 and 72.)

Epistylis umbilicata C. & L.

Body elongated oval, anterior end slightly constricted. Peristome not expanded. Ciliary disk has a central projection in the form of an umbilicus. Pedicle dichotomously branched, thick and very short, entire pedicle little longer than a single zooid. Colony composed of but few zooids.

This colony was found attached to an insect larva which, unfortunately, I did not attempt to classify. *Epistylis umbilicata* is said to occur on the larva of the gnat, *Culex pipiens*.

Length of zooids, 50 to 65 microns. (Fig. 75, plate XLVIII.)

Genus *Opercularia* Stein.

Colonial. Stalk branching and rigid. Zooids ovate and ellipsoidal. Ciliary disc attached to one side of the vestibulum, considerably elevated, and closing in a lid-like manner. A hyaline membrane projects from the inner border of the peristome. This genus was first described by Stein as having two sorts of zooids, but this was contradicted later.

Opercularia Sp.

Figs. 73 and 74 represent a species which has probably not been described. It seems undoubtedly to belong to the genus *Opercularia*. Stalk noncontractile, varying greatly on length and method of branching, which is irregularly dichotomous. The colonies vary greatly, from low, squat colonies of a hundred zooids to very slender zooids, like that represented by Fig. 74, often with not more than twelve zooids, the latter type being the more common. Individual zooids ovate, constricted beneath the peristome border, widest anterior to the middle of the body. Ciliary wreaths two, a knot-like projection on the center of the disc. Pharynx long and conspicuous, with a prominent pouch anterior to the middle of the body. Macronucleus oval to bandlike. Cuticular surface smooth. Membrane very hyaline and difficult to detect. Transverse folds and a few longitudinal striations usually visible at the posterior end of the zooid. Anterior end generally rounded when contracted, sometimes showing a snout-like projection.

The queer point about this form is the presence of zooids of two sizes. The small zooids resemble the large ones except for size. There is no regular arrangement of the small zooids, some colonies being entirely without them. From their irregular position on the stalk and the fact that they do not occur in groups, it seems clear that they are produced by unequal fission and not by multiple fission.

This form was found once in great abundance on leaves and sticks in a transient pond. I have not been able, however, to find it again, and for this reason have not described it as a new species.

Length of large zooids, 150 microns. Length of small zooids, 50-60 microns. (Figs. 73 and 74, plate XLVIII.

Genus *vaginicola* Lamark.

Body elongated, cylindrical, similar in general structure to *Vorticella* but enclosed in a transparent vase-like lorica. Zoöids attached to the base of the lorica either directly or by means of a short, thick stalk. Lorica sessile usually.

Vaginicola crystallina (?) Ehr.

Body considerably elongated, often containing chloryphyl-granules. Ciliary disc elevated. Contractile vacuole single. Macronucleus usually bandlike. Lorica nearly cylindrical, slightly narrowed anteriorly, rounded posteriorly. Body when fully extended projecting one-third of its length beyond the opening of the lorica.

The form pictured agrees with this species in all but size. Average length given for lorica, 119 microns. Length of type specimen, 55 microns. (Figs. 65 and 68, plate XLVI.)

Subclass SUCTORIA.

Infusoria which possess cilia during embryonic life only. Provided with tentacles for sucking or piercing during adult life.

Family PODOPHRYIDÆ.

Body usually spherical. Stalk present or wanting. Tentacles knobbed or pointed.

Genus *Podophrya* Ehr.

Body spherical, oval, or elongated, usually attached to some object by a rigid stalk. Suctorial tentacles knobbed, scattered promiscuously over the body or united into compact clusters.

Podophrya libera Perty.

Body spherical. Tentacles knobbed, long, from three to four times the diameter of the body, and scattered over the entire surface. Pedicle short and slender, about the length of the diameter of the body. Individuals often detached.

This species was found unattached in pond water. It seems to exist in this condition quite frequently, and it was originally described by Perty as an unattached species.

Diameter of type specimen, 45 microns. (Fig. 76, plate XLIX.)

Podophrya quadripartita C. & L.

Body elongated pyriform; anterior end forming four rounded lobes, each bearing a compact cluster of knobbed tentacles. Pedicle slender, somewhat dilated at its junction with the body. Macronucleus irregularly oval, often almost angular. Contractile vacuoles more than one.

Found in pond water, attached to *Epistylis*. One group was attached to a bit of leaf. Internal embryos are formed, and Fig. 78 represents a large individual with two such embryos.

Lengths given range from 85 to 200 microns. Length of type specimens, 75 and 143 microns. (Figs. 77 and 78.)

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CONTENTS:

THE DEVELOPMENT OF A TUNICATE WITHOUT NERVES, . . *Ida H. Hyde.*

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The Development of a Tunicate Without Nerves.

IDA H. HYDE.

From the Physiological Laboratory of the University of Kansas and the Woods Hole Biological Station.

A PRELIMINARY report is here presented of experimental work that deals with the differentiation of cardiac and other tissue independent of nervous control.

The organism employed was Ammarœcia, one of the Tunicates found in Woods Hole. It is viviparous, bearing the different stages of embryos in a definite sequence in the ova sack. When the notochord and motile tail are developed the larva swims out. It is active for about one hour, then attaches itself to the dish by means of its adhesive papillæ. In three days the heart begins to beat, and in four days the tail is absorbed and the organism is quite well developed. The nervous system, consisting of a vesicle formed of nerve cells and fibers, contains two pigmented sense organs. It functions before the pharynx, digestive system, and heart do.

In embryos the anlage of the tail, nervous system, and digestive tract can be recognized by the different-colored cells and their position.

For experimental purposes the embryos of different ages were easily dissected from the ova sack with fine steel points or hard glass fibers. Methylene blue and many other nerve

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stains and fixatives were employed in preparing the material for histological study, and camera drawings were made of some of the specimens.

At present only five sets of experiments were pursued, and for these controls were kept.

1. From a free-swimming larva the entire tail, with its notochord, nerves, muscles and tunic, was removed.

2. From young embryos the anlage of the tail was dissected.

The results from these two sets of experiments were that the organisms developed quite normally, but were smaller than the normal control.

3. Removed from a free-swimming larva the tail and also the nervous system with its sense organs.

4. Removed from young embryos the anlage of the tail and the nervous system, staining them, as well as the control, after four days with methylene blue.

The results from these two sets of experiments were that they developed much smaller than the control, many were abnormal, and in four or five days the heart began to beat and the siphons contracted when touched. The contractions were slower and not as complete as in the control, and at this time I can not state positively that the heart reverses its rhythm spontaneously.

5. Removed the nervous system and the heart anlage from young embryos. In some only part of the nervous system had been removed, as shown by the methylene-blue staining. The resulting cell complex developed into an abnormal structure devoid of heart and siphons, and with an incomplete digestive system. In those that retained part of the nervous system and pharyngeal cells, only one siphon developed. In others, in which the nervous system and part of the pharyngeal cells were removed but not the heart anlage, the structure regenerated its tunic, which contained, after four days, an abnormally formed digestive tract with a beating heart and one siphon.

We may conclude: that the cells which give rise to the heart, siphon and digestive tract continue to develop after the nervous system has been removed; the cells are independent living structures, capable of continuing their growth in a suitable environment, to a certain stage, but act slower and less completely to stimuli than they do in their normal coöperative medium; the fragmentary organism that is left forms an abnormal structure, with a protective tunic; the heart will beat and muscles differentiate without the presence of the nervous system.

Hooker, Harrison, Goldstein Shaper, Burrows, Carrel and others have worked on problems similar to mine. Their work will be discussed in the final paper.

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CONTENTS:

THE INFLUENCE OF LIGHT ON REPRODUCTION IN VORTICELLA.
Ida H. Hyde and Christine Spreier.

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VOL. XIX, NO. 15

The Influence of Light on Reproduction in Vorticella.

IDA H. HYDE AND CHRISTINE SPREIER.

From the Physiological Laboratory of the University of Kansas.

THE aim of the work briefly described in this article has been to ascertain the influence of monochromatic and different intensities of light on the reproduction of vorticella.

The vorticella were transferred by means of a Barber* capillary pipette, held in a modified triple-movement pipette holder.

From a culture of the unicellular organisms, that had been kept covered in the dark for several months, many encysted zoöids were isolated and transferred to a culture media, consisting of an infusion of Timothy hay. The infusion had been filtered, sterilized by boiling, and kept in sterilized sealed tubes until needed.

The encysted forms were under observation in a hanging drop placed in an open-end moist chamber.

As soon as the zoöids emerged from their cysts they were removed to a drop of the sterilized infusion placed on a Hemocytometer micrometer slide. By this means the direct increase in organisms could be ascertained. The slides with the young zoöids were placed in a Petri dish that contained a thin layer of water to prevent rapid evaporation of the solution. The organisms of the same age thus secured were kept for

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* Barber, M. A., Jour. of Infections Dis., 1911, vol. VIII, p. 348.

each series of observations, under the same conditions of temperature, moisture, food and intensity of light, but under different-colored rays, or where the intensity of light was studied under different intensities, for at least three days.

To ascertain the effect of colored rays, blue, green, yellow and red glass, gelatin films, and reflected rays from Hering's monochromatic colored cards were employed. The observations thus secured were compared with those made in sun or electric light of constant intensity. The colors were placed both in bright and dim light, and so that the reflected or transmitted rays penetrated the culture either from above or below. The heat rays were cut out by water contained in parallel-sided glass dishes placed in the path of the beams of light.

In order to study the influence of the intensity of light, the slides with the vorticella were placed either half or one and a half or two meters from a window having a southern exposure or electric light. Some were placed in a large blackened wall-box or in a box covered with smoked glass to vary the intensity of the rays of sunlight.

From the observations that were obtained from the various experiments, the following general conclusions were deduced:

1. Vorticella exposed to daylight for intervals of four days increase in number more rapidly on bright sunny days than on dark cloudy ones.

2. When the zoöids are placed at different distances from the source of light on an average 25 develop one-half meter, 10 one and one-half meter from the light, and 2 in the dark.

3. In comparing the greatest number that developed on sunny days from one zoöid in 24 hours it was seen that 40 grew under green, 29 under yellow, 26 under white, 13 under blue, and 17 under red.

4. The average rate of increase on cloudy days or under dim light was 4 for yellow, 3 for green, 2 for blue, white or red.

5. We conclude that the stimulating effect on the reproductive power of vorticella increases up to an optimum with increased intensity of light, and that the bright, luminous rays of yellow and green are more effective as stimulating agencies on the reproductive power of vorticella than are the red or blue rays.

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CONTENTS:

Some Methods of Studying Fossil Amphibia Embedded in Coal.
Roy L. Moodie.

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Some Methods of Studying Fossil Amphibia Embedded in Coal.

ROY L. MOODIE,
Associate in Anatomy, University of Illinois, Chicago.

Contribution from the Zoölogical Laboratory, No. 216.

Plate L.

THE study of fossils embedded in soft coal or shale has always been somewhat of a problem. Various means have been devised for the interpretation of the elements preserved, all of which have good points for the material in which the fossils are enclosed. The fossils embedded in coal are nearly always very fragile, or else only a mold of the skeleton is preserved, and the problem for the student is how best to obtain an idea of the form of the animal and still preserve the material for future investigators.

Jaekel (1) has devised a method by means of which the bones are removed from the coal, either by mechanical means or by chemicals, and impressions made of the mold either in wax, gutta-percha or plaster. He has been followed in this by his student, Schwarz (2). Their results are uniformly excellent, though their interpretations of the elements may, in some cases, be open to question. In regard to the methods of study Schwarz says:

"Der Erhaltungszustand ist im allgemeinen recht schlect. Dies gilt besonders von den amerikanischen Formen und . . . in der erwähten Arbeit betrachtet, an denen oft kaum die Umrisse

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zu erkennen sind, viel weniger irgend welche Einzelheiten der Organization. Hier konnte nur eine gründliche und sorgfältige Präparation zum Ziele führen. Es wurde dabei durchwegs-im Anschluss an die oft mit grossem Nutzen angewandte Methode Jaekel's der Knochen entfernt, wobei verhaltnissmässig gute Negative erhalten wurden. Eine grosse Erleichterung war dadurch ermöglicht, dass sich die Knochensubstanz in Salzsäure löst, während die Kohle nicht angegriffen wird. Die übrigbleibenden Teilchen des Knochens wurden dann mit der Nadel unter starker Vergrösserung entfernt.

"Fur die Abdrücke wurde zum grössten Teile Guttapercha dann aber auch Gips und in geringerem Masse auch Gelatine und Wachs verwendet." (p. 64.)

Much the same method has been employed by McGregor (3) in his study of *Meosaurus brasiliensis,* which was embedded in a black bituminous shale from the Permian of Brazil. He says:

"The matrix containing most of the remains is black bituminous shale, which splits rather readily in the plane of the specimen thus exposing, in some cases, almost the entire skeleton. The skeletons found in the bituminous shale are almost completely carbonized, and occasionally covered with a delicate pellicle of pyrite. They are much softer than the matrix, very friable indeed, and crumble badly on exposure to the air. Owing to this crumbling, it was found that the only way to study the remains satisfactorily was carefully to remove the carbonized bones and to take casts of the molds. From these natural molds or negatives, gelatine positives were first made, then, from these gelatine negatives, and from the gelatine molds final positive casts were made in plaster of Paris. Aided by careful comparison with the originals and with the gelatine positives, the plaster casts were colored to differentiate bone from matrix, and thus they illustrate the skeletal structure more clearly than do the originals."

The fossils studied by the writer were of such a nature that neither of the above methods was possible. This was due to the fact that most of the material was borrowed from the American Museum of Natural History, and the specimens representing the species are not sufficiently abundant to admit of preparation. Furthermore, a great many of the specimens studied were Cope's types, and as such, of course, could not be disturbed. Under these circumstances new methods of study had to be devised.

The fossils from Linton, especially, are all embedded in a soft coal, which is usually much broken, as if it had been picked up from the mine dump. The skeletal elements in all

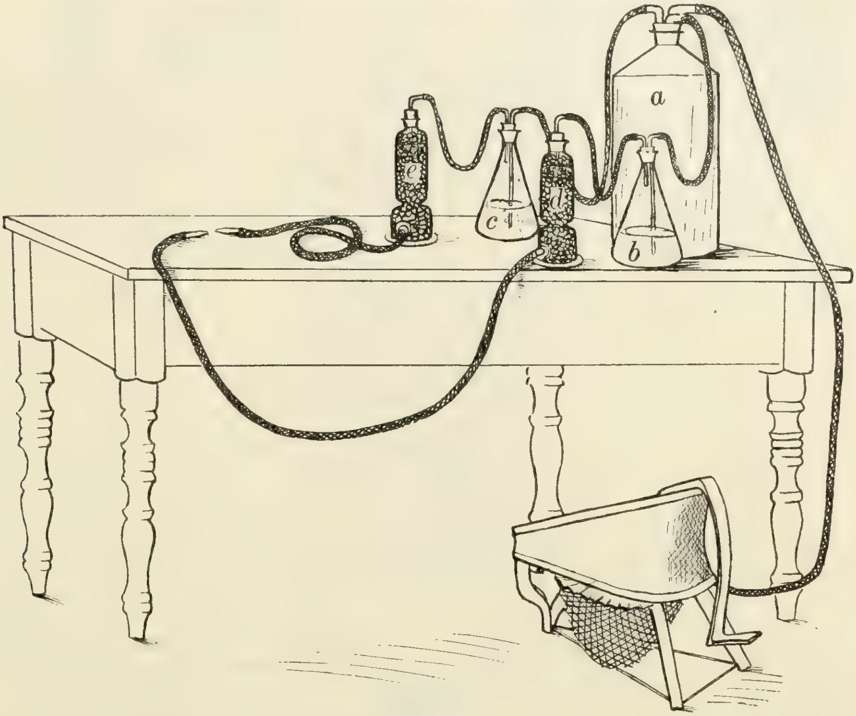

FIGURE 1.

1. Apparatus for whitening dark or black fossils in order to bring out details for photographic and study purposes. A foot bellows with a long tube attached through which the air is forced into the reservoir *a*. From this reservoir pass off two tubes leading through two wash bottles, *b* containing C. P. HCl, *c* containing C. P. NH_4OH. The fumes from these are carried through drying towers containing chloride of lime, *d* and *e*, to take out all moisture; then the fumes are mixed at the tips of the glass canuli, forming a white deposit of ammonium chloride (NH_4Cl) on the object to be studied or photographed.

cases are completely carbonized and crushed flat. In some cases a thin carbonaceous pellicle adhered very closely to the fossils, and this was difficult to remove. The Linton fossils are all black, and can only be detected by close scrutiny in certain lights. The important thing was to treat the specimens so that the fossils would be distinct in ordinary lights. This was done by an apparatus (figure 1) which deposited ammonium chloride in a tenuous layer over the fossils. The apparatus consists of a bellows, or a mouth tube, to blow air through the tubes; two wash bottles, one containing chemically pure ammonium hydroxide, the other with chemically pure hydrochloric acid; and two drying towers filled with calcium chloride to extract all moisture from the air.

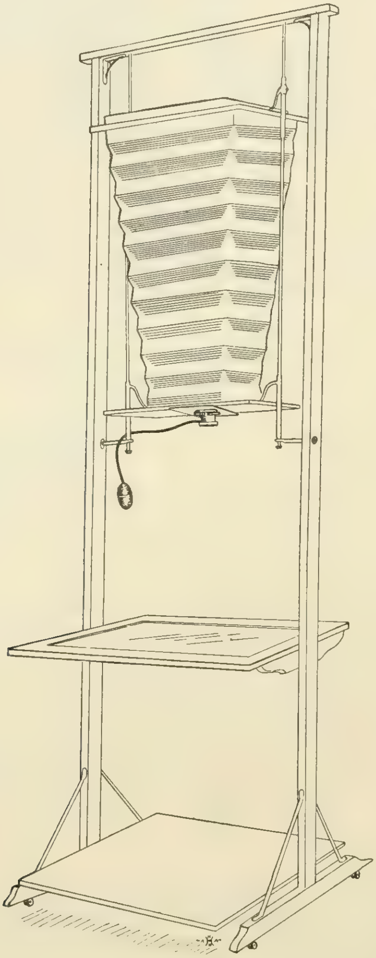

FIGURE 2.

A vertical camera designed especially for making photographs of large delicate objects. By means of a clear-glass background shadows are eliminated. This camera was designed and made by Dr. S. W. Williston.

The fossil could then be photographed and the photograph used as an illustration, or, if the details were still obscure, as an aid to studying the specimen under the binocular. The apparatus which has been found of the greatest convenience in photographing the smaller fossils (figure 3) consists of a heavy iron base into which is fastened an upright steel bar an inch and a quarter in diameter, to which is fastened, by means of clamps, a base and a rod for the support of the camera.

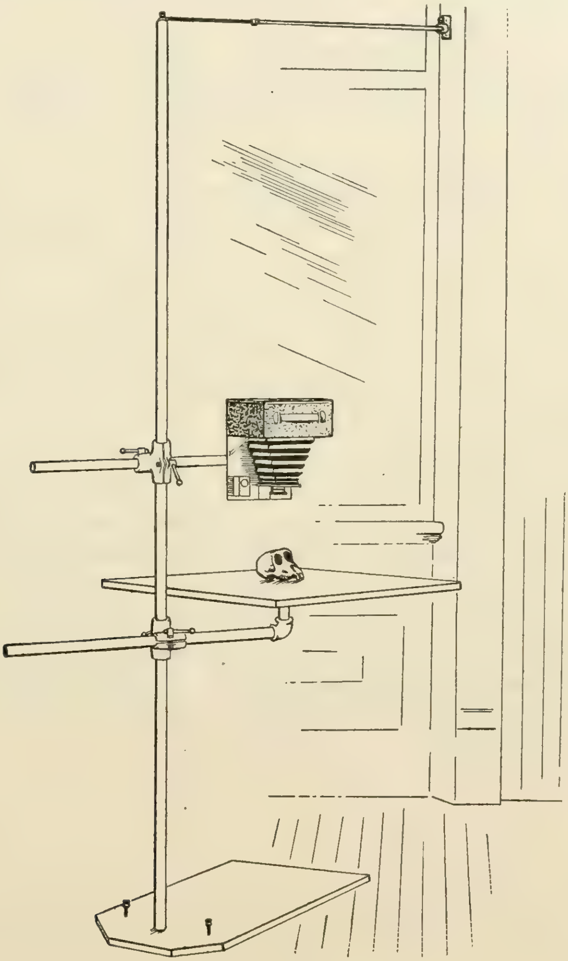

FIGURE 3.

A vertical camera stand, useful in making photographs of small objects.

Either base or camera can be moved at will up or down the vertical rod. Another type of camera which is of great value, especially for larger fossils, is the one shown in figure 2.

Prints made from the negative are often used for illustration, but in case the photograph is not clear enough for publication, outlines are taken either from the print or from the negative. The fossil is then placed, with the coating of ammonium chloride still adhering, under a Zeiss binocular where, under magnifications of from 8 to 50 diameters, the characters of the fossil are studied in connection with the photographic print. The lower magnification was found to be the best in most cases, for no object is even slightly distorted by this magnification, and the structures stand out with surprising clearness and beauty. This is especially true when the light from a fifty-candlepower electric lamp is cast obliquely on the fossil while under observation. In cases of finer structures, such as the muscular fibers in the body wall of *Tuditanus walcotti,* under the 50-diameter magnification, the light was indispensable. On the outlines made from the photographs the details of structure are put in while the object is still under observation with the binocular, so that the chances of error have been reduced to the minimum.

The above remarks apply especially to the fossils from the Linton, Ohio, coal measures and those from the Cannelton slates. The Mazon creek nodules are very simply studied for they are usually clearly preserved, but the Zeiss binocular was found of the greatest service. The bones in the Mazon creek specimens have all been transformed into kaolin. The bones, in some instances, have been removed and wax casts made of the impressions. This method was used by Doctor Hay (4) in studying a specimen of *Amphibamus grandiceps,* and I have found it very useful.

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CONTENTS:

THE EFFECTS OF PHYSICAL FATIGUE ON MENTAL EFFICIENCY.
Floyd Carlton Dockeray.

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The Effects of Physical Fatigue on Mental Efficiency.

FLOYD CARLTON DOCKERAY.

OUTLINE.

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INTRODUCTION.

THERE are three possible effects of physical fatigue upon mental efficiency: (*a*) It may produce decreased mental efficiency, either in the speed or accuracy of the work, or in both; (*b*) it may serve as an incentive to greater effort and

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thus increase the efficiency; or, (*c*) it may have no appreciable effect in either direction. Furthermore, it might be assumed that all these effects might be manifested in the same individual under different physical conditions, or that, given a definite amount of physical fatigue, individuals might be divided into groups according to the effects produced. It might also be supposed that the immediate effects would not necessarily be the same as those to be noted later, say in a half hour, or even after a longer time. The immediate effects of physical fatigue on mental efficiency are all with which we are directly concerned in this paper. Though the later effects may be of even greater importance, the amount of time involved in such tests has made it impossible to collect sufficient data to warrant their incorporation here.

HISTORICAL.

As Max Offner (31) has reviewed the literature dealing with the various phases of fatigue as they affect the problem of school hygiene, and as C. D. Yoakum (50) gives a very thorough review of the general field, it is not deemed advisable to attempt a discussion of the subject here, except those phases which bear more directly upon the relation between physical and mental fatigue.

Mosso (30) seems to be the first to call attention to the possible relation of physical and mental fatigue. He found with the ergograph which he invented that after voluntary contraction of the finger was no longer possible the finger could still be made to lift the weight if an electric stimulus was applied to the flexor muscle. If the subject was mentally fatigued by lecturing, or by a two-hour examination, the amount of voluntary work he could do on the ergograph was decreased, and the muscle failed to respond to the same extent to the electric stimulus that it did before mental fatigue. It would appear, therefore, that mental fatigue had a direct influence upon the voluntary muscles. However, not all of Mosso's subjects were in complete agreement. Under normal conditions part of the subjects produced convex curves and part concave curves. Only those with concave curves showed reduced muscular ability immediately after mental work. Those with convex curves showed an increase immediately after the mental work, followed an hour later by a decrease.

Mosso considers this difference to indicate merely different types of individuals. Mental work causes not only fatigue but a heightened emotional state, which endures longer with one class than with the other. This heightened emotion causes the greater muscular activity in some individuals. As an explanation of the fact that mental work does have an influence on the ergographic tracings he suggests two possibilities: (1) The muscles might cede a part of their nutriment to the nervous elements, as in fasting. But the muscles rapidly recover after the fast is broken, while the weakness known as fatigue requires time and sleep. (2) There is a fatigue product generated which circulates in the blood and makes fatigue general. This he demonstrated by the transfusion of blood from a dog that had been kept running for several hours into a dog that had rested for the same period. The second dog showed all the signs of fatigue manifested by the first dog. The work of Mosso has been somewhat criticized by later investigators. The amount of physical exercise involved in lecturing is a very important factor which no doubt would affect the ergographic results, but which he neglects to take into consideration. Also, his tests were not repeated sufficiently to secure a constant tendency under a particular set of conditions.

Lombard (24), working with a Mosso ergograph, found that the voluntary contraction of the muscle gave rhythms of contraction which still appeared when the muscle was massaged. When the muscle was caused to contract by the application of an electric stimulus until it could no longer lift the weight, it would still respond to a voluntary stimulus. Furthermore, when one finger was fatigued by voluntary contraction, another finger would give as great a curve as before. He also found that in some individuals there are periods of muscular ability and intervals of inability which succeed each other more or less regularly after the first period of fatigue, even though the will is applied uniformly at the maximum throughout the experiment. These later changes did not appear when the nerves or muscles were stimulated electrically. He concluded that while the fatigue from voluntary contraction must be of central origin, it must lie between the "will center" and the centrifugal nerves. The central origin of fatigue was also evidenced by Joteyko's (17) ergographic studies, though she found the end plates in the muscles fatigued first. In her experiments fatigue of the right hand decreased the power of the left, which would seem to be a slight contradiction of Lombard's results.

Since these early ergographic studies the possibilities of the application of the ergographic curve as a means of testing mental fatigue has been assumed by many investigators of the problem in the public schools with very contradictory results. Kemsies (20) found, for example, that (1) Monday and Tuesday are the best days of the week; (2) the first two hours of the day are the best of the day, except on Monday; and (3) vacations of not more than three weeks show a beneficial influence in proportion to their length. It was believed that the mental work of the schoolroom produced the decrease in the ergographic tracings, and that there was not complete recovery from one day to the next.

Bolton (3) tested the method more thoroughly in connection with other tests after both physical and mental fatigue. His results for five tests each showed that the performances with the ergograph were considerably increased after two-hour periods of adding, decreased after two-hour walks, and remained unchanged after two-hour rest periods. However, he was not satisfied that the ergograph was a reliable test. Keller (19), on the other hand, used the same method to test the fatigue induced by rapid reading, and found slight indications of fatigue. Ellis and Shipe (11) repeated Keller's experiment, with the result that, while reading showed fatigue, it was not evidenced by the ergographic curves.

The dynamometer has been used frequently as a more convenient method of determining muscular ability after mental work, but the results are even more variable than those obtained by the ergographic method. This may be due largely to the purely accidental factors that may enter into so brief a test. The physical and mental adjustments at the moment are liable to greater variation than would be possible in a longer test. It is also possible that the subject is able to concentrate his effort for the moment required, and obscure the fatigue effects. Direct correlation between mental fatigue and the dynamometric readings was shown by Binet and Henri (4), and their results were confirmed by Clavière (8). The latter divided school children into three groups. To the first group he gave difficult lessons; to the second group, easy lessons; and to the third group, rest for the same period. The tests correlated with the groups. Thorndike (38) repeated similar tests, but found no correlation.

Reaction times and the spatial threshold as tests of fatigue have been used, but the greatest fault with either method is the great difficulty in determining what function is measured. Considerable dispute has arisen as to whether change in reaction time is a function of a change in the sensory or motor process or in attention. Scripture (34) found that "fatigue in reaction time increases with the complexity of the adjustments required for perceiving the stimulus. There is least fatigue when only an act of attention is involved." (p. 19.) Milroy (27) used one subject on continuous reactions, and found longer reaction times in the last half of the interval, but changing the sense organ did not interfere with the retardation.

The most careful study of physical fatigue by means of reaction times was done by Bettman (1). Unfortunately he was the only subject for the experiments. He performed the experiments, with the aid of an assistant, during vacation, under as uniform conditions, so far as diet, sleep, etc., are concerned, as is possible. The tests consisted of fifty choice reactions before and three hundred choice reactions immediately following the work or the rest periods. He either walked two hours, added one hour, or rested one hour. The results showed an increase in reaction time after mental work and a decrease after physical work, though the greater number of false reactions in the latter case is considered as evidence of decreased mental activity after physical work also. He also employed in place of the three hundred choice reactions a combination of fifty choice reactions, a half hour of adding and a half hour learning nonsense syllables. In these tests, also, he found a decrease in mental activity after either form of work.

The æsthesiometer method has been more widely used as a test of fatigue, but is open to some of the same objections as the reactions test. The chief fault, however, is the brevity of the test as frequently used. Griesbach (13), for example, was able to examine six to ten subjects in ten minutes. Any one familiar with the two-point threshold experiment would expect variations that would obscure the real character of the results in a test that required only one minute. Bolton (3), in fact, worked with the method in Kraeplin's laboratory, and found marked variations which he ascribes to after-images, tingling sensations and the varying sensibility of the neighboring portions of the skin. These variations obscured any possible evidence of fatigue after adding one hour or walking two hours. Nevertheless, Griesbach (13), Wagner (44) and Vannod (41) have obtained results which they are satisfied show a definite increase in the spatial threshold of the skin after work, principally after gymnastic exercise; and in a later investigation Griesbach (14) has not only considered this a measure of fatigue, but he finds that, while the spatial threshold for both sides of the body may be increased with fatigue, the increase is greater on one side or the other, depending on the nature of the exercise. Thus, for physical exercise, he finds a decrease in sensitivity of the left side of the body, or a decrease in discriminability of the right hemisphere. The experiments were performed upon forty-six soldiers at six to seven o'clock in the morning. The soldiers then marched several miles to their drill grounds and spent the remainder of the time until ten o'clock in general gymnastic exercise. The men were taken from the field, one at a time, and again tested. The pulse rate was found to be much higher and the average threshold value on the left side of the body was greatly increased for every subject. The average increase for the right side was only slight, and many subjects showed decreases.

The chemical products of fatigue are of particular interest to our problem. The presence of sarcolactic acid and potassium with fatigue easily leads to the assumption, as similar phenomena did for Mosso (30), that their circulation in the blood must cause general fatigue. It may be that some day the physiologist can demonstrate the presence or absence of these substances in the cerebral centers following muscular fatigue. At present the experiments are confined to their effect upon the muscles and motor fibers. As early as 1865 Ranke (33) found that the injection of a small amount of lactic acid increased and a larger amount decreased the irritability of an isolated muscle. Recently these experiments have been repeated more extensively by Lee (21) and by Burridge (6). By the introduction of fatigue substances in small amounts for long times, or in larger amounts for shorter times, Lee produced an increase in the height of the work curve. Still larger amounts produced a decrease at once. Burridge's results lead him to believe that "a quick recovery is to be associated with potassium salts alone or in excess while a slow and prolonged one has only been obtained as an effect of lactic acid. It has also been shown that the effect of an exhausting tetanus is almost identically the same as that found to occur when a high concentration of lactic acid has been perfused through a muscle. There is thus reason to believe that the results noted in the early stages of fatigue are mainly referable to potassium and those of the later stages to lactic acid." (p. 303.) The results of these investigations, particularly Lee's, conform to what is generally observed in the fatigue curve of an isolated muscle, or the curve from voluntary contraction. Hough (16) and Lee show that the fatigue substances also affect the nerve fibers as well as the muscles.

The relation of the fatigue products to mental fatigue has been little studied. Lehmann (22) measured the amount of carbon dioxide exhaled during mental work. He found that mental work of a determined kind and amount has, with the same individual, a constant increase in the amount of carbon dioxide and corresponds to a definite energy measure.

To summarize, we find the relation of physical and mental fatigue much in dispute. Mosso finds fatigue general; Lombard considers it special, but of central origin; while Joteyko's work would indicate that it is partially of central origin and more or less general in character, through the fatigue of the voluntary center. Many of the other tests have been too carelessly performed, or by their nature have been inadequate, to throw much light on the subject. The only experiments which in any way correspond to the experiments to be reported are those of Bettmann and of Bolton. The former, unfortunately, tested only one subject. The latter, though his work was thorough, aimed to test methods that he considered unreliable, and consequently his work throws little light upon the relation of physical fatigue to mental efficiency.

METHODS AND RESULTS.

The experiments were undertaken in the psychological laboratory of the University of Kansas in 1910, and continued until 1915. During the summer the work was carried on at the University of Michigan. The author is indebted to Professor Pillsbury for suggesting the problem and for many suggestions relating to the procedure. Especially deserving of gratitude are those who have given so faithfully of their time and energy as subjects for these experiments, which were of necessity decidedly irksome. The subjects in the University of Kansas laboratory were Professor D. C. Rogers, E. D. Campbell, instructor in logic, and George Babb, Raymond Edwards, William Hoyt, Verne Miner, Emily Berger, George Bunn and Carl Brown, all advanced students in psychology, except Mr. Bunn, a sophomore engineer, who was employed in the laboratory as mechanic. In the Michigan laboratory Professor John F. Shepard, and W. H. Batson, R. I. Cook, J. E. De Camp, and C. S. Wang, graduate students in psychology, were subjects.

ADDITION TEST.

The first test to be used was the addition of figures. The test differed from the Kraepelin tests in that the numbers to be added were in single columns of twenty numbers each, and the subject added the whole column and wrote the sum at the bottom. In the Kreapelin tests the subject added only two numbers at a time. He might, or might not, write the sum. There are two advantages in this method: (1) it is slightly more difficult to add and retain the sum of twenty numbers than it is of two numbers as in the Kraepelin method; and (2) it provides an easy means of determining the accuracy as well as the speed of the operations without any serious interference from writing the sums. In fact it was found that the subjects soon acquired the habit of beginning to add the second column before the answer to the first was entirely written. All subjects were given a few days' training, though not enough to render further improvement negligible, for it was believed that, while the subjects should be familiar with the method, it was not necessary to eliminate improvement entirely. The tests, of fifteen minutes' duration, preceded and followed an interval of fifteen or twenty minutes. The validity of a test of this length will be discussed later.

Tables I*a* and I*b* show the results obtained from three subjects. Ba. and Da. were strong, healthy men, accustomed to all sorts of gymnastic exercises, including running, swimming and tumbling. Ca. was decidedly of the opposite type, and unaccustomed to any sort of robust exercise. Accordingly the same physical work done by him should represent more fatigue. The physical work done by Ca. and Da. consisted in a uniform exercise with the ordinary wall machine of the gymnasium. The subject stood with feet together and bent the back until the hands were on a level with the knees. From this position he rose to the erect position with the hands above the head. This movement was repeated thirty times per minute for from five to twenty minutes. The weight varied as indicated in Table I. Ba. ran various distances on the indoor track. These distances were not run at the subject's best speed, but at a fairly fast rate, which did not vary materially throughout the interval. This uniformity was maintained by the subject setting a standard rate which he considered almost his best for the distance to be run. At the end of each lap he was informed as to whether he had run faster or slower than the standard that had been established.

MULTIPLICATION TEST.

It was soon found that for most subjects at our disposal the simple addition of single figures was inclined to become so automatic that it was doubtful if it required enough attention to be considered a good test. Frequently a subject would report that he heard conversation in the adjoining rooms, but did not feel disturbed in his work. He might even give slight attention to other subjects without affecting the results sufficiently to be detected in the records. For this reason it was considered best to substitute a test that would demand the most careful attention on the part of the subject, and at the same time would not require elaborate apparatus. After the consideration of several tests "cross multiplication" was chosen. The subject was given a series of problems of two or three place numbers, one below the other (the digits 1 and 0 were never used), and instructed to multiply as rapidly as possible and still be accurate. He was allowed to record the partial final product as it was obtained. That is, in such a problem as $\begin{array}{r}374\\ \underline{568}\end{array}$, the operations were performed as follows: 8×4, record 2; $(8 \times 7) + 3 + (6 \times 4)$, record 3; $(8 \times 3) + 8 + (5 \times 4) + (6 \times 7)$, record 4; $(6 \times 3) + 9 + (5 \times 7)$, record 3; $(5 \times 3) + 6$, record 21. The problems were not so difficult, therefore, as in Thorndike's (37) tests, but the subjects were able to perform more of them in a given length of time. Many of my subjects, moreover, found it next to impossible to retain the total product until the entire problem was finished.

It was difficult to determine what should be considered the unit operation for computing the mistakes. The units place in the total product involves one multiplication, the tens place involves two multiplications and one addition, and so on. Furthermore, a mistake in the tens place might show itself in the hundreds and not in the tens place at all. It was finally decided to consider a problem wrong that was not entirely correct. While this method allowed rather wide variations that could not be recorded, it seemed to be as nearly accurate as any other method that could be devised.

In order to test the effect of the fatigue of a small group of muscles as compared with the more general physical fatigue of the whole body, or at least of a much larger group of muscles, two types of ergographs were used. One consisted of a small spring about six inches long suspended between two vertical bars. Beneath the spring hung a brass band into which the thumb of the left hand was inserted. The whole ergograph was mounted on a small board raised to the level of the arm-rest. The arm was strapped to the arm-rest, palm up, with the fingers under the board supporting the ergograph. A downward pressure with the thumb, therefore, was accompanied by contraction of the flexors of the four fingers as well as of the thumb. The principal fatigue, however, seemed to be felt in the muscles controlling the thumb. The contractions were recorded on the kymograph by means of a marker attached to the spring. The more general fatigue was secured by means of a heavy spiral spring mounted in an iron tube about two feet long and three inches in diameter. The tube was attached in the vertical position to a plank base two feet square. A heavy rod was attached to the lower end of the spring and projected about a foot above the top of the tube. The upper end of this rod was threaded so that a handle could be adjusted at any height desired. A pointer projected through a slit in the side of the tube and indicated in hundred pounds the tension on the spring. A thread ran from the lower end of the rod through the base, under two pulleys and up through the edge of the base. This thread could be attached to the marker that recorded the results on the kymograph. This furnished a somewhat different sort of exercise from the weights used in the earlier experiments. In place of the long sweeping motion in swinging the arms from the knees to above the head, an exertion of two hundred pounds caused a movement of the handles of the ergograph of about five-eighths of an inch.

Co. and De. were tested at the same hour, one with the thumb ergograph and the other with the body ergograph. The next day their places were reversed, and on the third day both rested between tests. Do. worked with the wall machine, the ten-minute work periods being begun first and the twenty-minute periods undertaken as the subject felt capable of the longer period. Consequently the twenty-minute periods do not represent exactly twice the fatigue produced by the ten-minute periods, though they alternated with the later ten-minute periods. As Do. worked alone, it was necessary to arrange some sort of a signal to announce the end of the test period. It was found that a stop-watch directly beside the problems on the table was satisfactory. A glance at the watch would indicate the time. While this interfered somewhat with the last problem or two, it was believed the interference was slight. However, it is well to keep this difference in method in mind when considering his results. It was not thought wise to attempt the subdivisions into five-minute periods. The physical work of Ba. was similar to that with the addition tests, supplemented by cross-country running, which was over hills and rough roads and was considered by the subject more fatiguing than a longer distance on the indoor track. The results are shown in Tables II*a* and II*b*.

SOUNDER TEST.

A simple attention test was planned for the third series. Pillsbury (32) has suggested the attention wave as obtained with the minimal light as a measure of fatigue, and MacDougall (26) has proposed the marking of dots as they appear through an apperture in irregular order. The difficulty with the latter is that it involves a large proportion of motor activity to the degree of attention required. While the attention wave with the minimal light has in general proved satisfactory in the Michigan laboratory, special conditions in the present series made an alteration of the test seem advisable. The sounder test was first suggested by Professor Shepard. Four telegraph sounders were selected with as nearly the same quality of sound as possible, and adjusted to approximately the same intensity. A short piece of rubber tubing was slipped on the end of the hammer of each sounder to render the back stroke inaudible. In parallel with each sounder was a recorder and a rheostat. The recorders were adjusted to write less than a quarter inch apart (in later experiments, one-eighth inch apart) on the smoked paper of the kymograph. A fifth recorder wrote in the same vertical line with these. It was connected with the subject's key and recorded his reactions. The sounders were operated by four keys arranged in a convenient row. The subject to be tested sat at a table several feet from the sounders, with his hand upon his key. The experimenter operated one of the sounders several times in rapid succession (six times in two seconds) as a signal. He then operated the four sounders one second apart in irregular order for one minute. The subject was to react to the "signal" sounder, whenever he recognized it, by pressing his key. At the end of the minute the operator signaled with another sounder, to which the subject was to react for the next minute. This was continued for sixteen minutes, the four sounders being used as the "signal" sounder once in each four-minute period, but not in the same order.

The records were preserved upon smoked paper belts about ten feet long. As the markers wrote closely together and the paper traveled fairly fast, and thus spread out the marks horizontally, it was an easy matter to count the number of omissions and errors (wrong reactions). The signal at the beginning of each minute required two seconds. There were, therefore, fifty-eight clicks each minute. The one exception was in the records of Do. The experimenter in this series did not use a stop watch or metronome as a guide, as in the other series, but operated the sounders at a convenient rate, which was somewhat faster than one per second. In all the series the effort was made to make the order as irregular as possible, and at the same time sound each sounder approximately an equal number of times. It was found, however, that sounder number three (operated with third finger) got the preference, followed by two, one and four. There was also a difference in the difficulty with which the sounders were distinguished. Numbers two and three were most difficult and most often confused with each other.

As might be expected, some subjects could distinguish the sounders more easily than others. Thus the test was found to be very easy for some subjects and difficult for others. Necessarily it would be as poor a test for either extreme. The sounders could not be adjusted for each subject. The method finally hit upon was the introduction of a fifth sounder which should sound simultaneously with each of the others, and the intensity of which could be adjusted readily. The best means of making this adjustment was found to be to adjust it so as to obscure almost completely the difference in the other sounders. Then, by inserting one or more thin papers under the hammer, the intensity could be reduced and the differences in the other sounders made more distinguishable. For some subjects two papers were used; for others one paper was sufficient, and for one subject, De., no paper at all was necessary.

The average number of clicks in each four-minute period to which the subject should react is given in the first column of Table III*a*, followed in the second column by the average number of times he did not react, and in the third column the average number of times that he reacted wrongly. Though in reality both failures to react and wrong reactions are errors, for the sake of brevity we will speak of the former as "omissions" and the latter as "errors." The quality of the work done is expressed in this series negatively, that is, an increase in per cent means a decrease in efficiency, as it is the mistakes instead of the successes that are recorded. The per cent of omissions and the per cent of errors, given in the fourth and fifth columns, are based on the first column, though the per cent of errors might just as reasonably have been based upon the average of all sounders, that is, 232. The totals given in Table III*a* are computed directly from the corresponding columns of the four periods into which the tests were divided, while the results shown in Table III*b* were determined from the totals of the individual daily records. The two sets of results do not, therefore, agree perfectly, but whenever there is any divergency, Table III*b* should be considered the more exact. In Table III*b* the per cent of increase or decrease in the second test is determined by dividing the per cents of omissions and errors of the second test by the corresponding number of the first test.

Two of the subjects walked between tests for two to three hours' traveling distances ranging from nine to fifteen miles, as recorded by the pedometer. As these walks occurred but once each week, and as neither subject was accustomed to much walking, they were very fatiguing. The other six subjects used the big spring ergograph already described. However, it had been found that the subjects suffered so much from sore hands after the first day that they were unable to lift sufficiently hard to render themselves very fatigued. Accordingly a harness was introduced, which consisted of a padded strap over each shoulder and attached to tugs in front and behind. The subject stood astride the ergograph and the tugs were attached to the handles of the ergograph and shortened sufficiently that the subject could stand erect only when he lifted his maximum amount. This meant that he was always under strain, for when he was not lifting, he was standing in an awkward position that was in itself fatiguing.

ASSOCIATION TEST.

In the association tests nonsense syllables were used, and the method was the usual method of successes. The apparatus used to expose the syllables was the Wirth form made by Zimmermann, slightly modified. Ten pairs of syllables usually constituted a series. As there are fourteen spaces in the cylinder of this apparatus, this left four blanks. The syllables were always arranged so these blanks came together at the end of the series. In the case of one subject, R., these blanks were filled, making fourteen pairs of syllables. Four seconds, counted by the metronome, were allowed for each space, including the blanks. The number of repetitions of the series was determined for each subject by preliminary tests. An approximate average of seventy-five per cent for the normal score was chosen, but in some cases it was found necessary to accept a lower score, as some subjects required more time than was available to reach the higher score. The number of repetitions was, therefore, twenty-five or thirty, but always the same number for any given individual. The tests of recall were always made the next day, after a definite interval, which was always uniform for a given individual, though for some it was not twenty-four hours. The Hipp chronoscope, prepared for a make-and-break circuit, was connected with the memory apparatus to measure the time between exposure and response. In calculating the per cent of correct responses, all syllables which had formed sensible associations were discarded, except for one subject, R. In his case it was found practically impossible to learn nonsense material when he did not form sensible associations with the syllables. An examination of his records shows that he was fairly constant, when given freedom to associate the material as he chose. I therefore feel that it is allowable to include his records, though they will deserve separate consideration. After discarding some of the results, as mentioned above, those that remained were used as a basis for determining the per cents. Those responses that had one letter wrong were considered equal to one-half, and those with more than one letter wrong were valued at zero. In a few cases a syllable might be completely reversed. If the first and last consonants were very similar, such responses were evaluated one-half. This occurred in only a very few instances. The average time for the responses was calculated from those responses that were considered wholly or partially correct.

Two of the subjects, Do. and H., learned the series Sundays at 11:30 a. m. and tested the next day at 7:30 a. m. Subjects Br., R. and E. learned at 3:30 p. m. twice a week, and tested the following days at the same hour. At least one day intervened between one test and learning the next series. Part of M.'s work was done at 10:30 a. m., and an equal amount at 4:30 p. m., but always the period was preceded by an hour of rest from any strenuous work, and an equal number of rest and fatigue days were given at either hour. His tests were twenty-four hours later. The fatigue exercise consisted of long walks of from nine to fifteen miles for Do. and H., twenty minutes in the harness for Br., R. and M., and five-mile runs for E. The results are given in Tables IV*a* and IV*b*.

Throughout all the tests an effort was made to preserve a reasonable regularity in the subject's general condition. By "reasonable regularity" is meant the average normal condition of the individual subjects. No restraint was put upon a subject who was in the habit of using tobacco or other stimulant, other than to require that he should not alter his habit preceding a test. It was considered that a man who smokes would be more normal if allowed to smoke as usual than he would be if this were prohibited temporarily. The days for the tests were chosen that were freest from fatiguing occupations for each subject concerned. If for any cause a subject did not feel that he was normal on a certain day, the test was deferred. While some tests were taken with some subjects in the late afternoon, they were always preceded by rest or a day of light work, which was fairly constant for the different days.

METHODS OF TABULATING RESULTS.

Tables I*a*, II*a* and III*a* give the averages of results by periods of five or of four minutes. It would be reasonable to expect that where averages are given, some means of showing how nearly the individual results conform to the average would be given also. The inconsistency of stating an average without stating the mean variation, at least, is recognized. But, granting the inconsistency, it is believed that these tables justify their presence in this discussion. The reasons for the incompleteness of these tables are: First, neither averages nor mean variations of the periods from day to day are very important for our purpose. We are interested principally in the differences between the forework period and the afterwork period of the same day. In the second place, these tables are already large. The addition of mean variations for every average would make them too confusing. A further discussion of the results of these tables will be found in the discussion of the curve of work (p. 236). Tables I*b*, II*b* and III*b* present the totals of results for each day, and the per cent of increase or decrease in the second test when compared with the first. If the effect of rest or physical fatigue is the same each day, then these per cents would be the same. The average and mean variation is therefore legitimate in this case.

In the association test it is necessary to compare the results obtained on the various days. For this reason the conditions on the various days was kept as nearly uniform as possible.

The average time of recall for each day is given in Table IV*a*, but instead of giving the mean variation, the number of responses that required more than 10,000 σ or less than 3000 σ are given in Table IV*b*. The averages in this table were obtained from the total number of individual responses, while the final averages in Table IV*a* were obtained by taking the average of each day's average.

In case any one should wish to make a more careful study of the results than these tables permit, a complete duplicate copy of all the results used in compiling these tables may be procured from the general library of the University of Michigan or the University of Kansas.

RESULTS.

TABLE I*a*.—ADDITION TESTS. Average number of problems, and per cent correct, from total daily records given in five-minute periods. (For number of cases, see Table I*b*.)

Subj.	Interval.	Test.	First 5 min.		Second 5 min.		Third 5 min.		Total.	
			No.	% Cor.	No.	% Cor.	No.	% Cor.	No.	%Cor.
Ca.	Rest 15 min.	1	7.1	83.8	8.8	55.2	7.5	76.9	24.3	69.6
		2	8.4	90	9.5	56.4	9.3	36.8	27.8	66.4
	5½ kg. 7½ min.	1	8.6	74.4	8.4	35.9	9.6	59.3	26.6	59.2
		2	9.3	67.7	8.4	82.2	8.3	90	26	67.4
Da.	Rest 15 min.	1	11.7	83.7	10.9	72.3	9.7	81.4	32.4	77.4
		2	12	76.8	11.2	58.4	11	75.8	44.3	71
	5 kg. 10 min.	1	10.9	60.8	10.3	51.4	11.9	71.2	33.1	61.1
		2	12	60.3	10.7	81.9	13.1	71.7	36	70.7
	10 kg. 10 min.	1	12.7	80.5	11.5	88.6	11.9	75.2	36.1	80.4
		2	13.1	82	12.1	81.4	11.2	69.7	36.5	78.8
Ba.	Rest 15 min.	1	8.1	78.7	7.8	71.9	8	89.6	24	79.9
		2	8.7	67.4	7.4	73.8	7.4	96.4	24.2	74.8
	5 kg. 10 min.	1	7.5	77.9	6.5	79.6	6.9	82.4	20.9	79.6
		2	7.9	74.6	7.1	77	7.5	70.7	25	73.8
	9 laps, 167 sec.	1	10.3	83.9	8.8	88.6	8.9	88.8	28	87.1
		2	8.9	76.7	8.1	87.5	8.5	80.1	25.5	81.4
	18 laps, 360 sec.	1	10.5	80.9	9.7	84.8	9.3	94.1	29.5	86.6
		2	8.5	95	9.3	73.5	9	89	36.8	85.8
	27 laps, 567 sec.	1	9.5	88.4	9	90.6	9.5	88.5	28	91.2
		2	10.5	94.8	9.7	80.8	9.3	88.9	29.5	88.1

TABLE I*b*.—ADDITION TESTS. Total results from each day's record and the per cent of increase or decrease in the second test. The first test is considered 100%.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	No.	% Cor.	Interval.	No.	% Cor.	No.	% Cor.
Ca.	19.5	52.6	Rest 15 min.	23.5	65.9	120.5	125.3
	27.5	85.7	“ “ “	30	63.3	109.1	75
	27	70.4	“ “ “	30	70	111.1	99.4
					Av.	113.6	99.9
					m.v.	4.9	16.9
	25	54.2	5½ kg. 5 min.	24	83.3	96	153.7
	23	34.8	5½ “ 7½ min.	24	39	104.3	112.1
	32	87.5	5½ “ 10 min.	30	80	93.7	91.4
					Av.	98	119
					m.v.	4.2	23
Da.	36.3	77.9	Rest 15 min.	37.4	83.9	103.1	107.7
	24.4	68.5	“ “ “	31.5	46	129.1	67.2
	35.5	85.9	“ “ “	34	83.3	95.8	97
					Av.	103.9	90.6
					m.v.	13.1	15.6
	27.6	65.2	5 kg. 10 min.	30.4	66.9	110.2	102.6
	39	57.9	5 “ 10 “	37.7	60.2	96.7	104
	32.7	60.2	5 “ 10 “	40	75	122.3	124.6
					Av.	109.7	110.4
					m.v.	8.7	9.5
Ba.	17.8	67.4	Rest 15 min.	19	78.4	106.7	116.3
	22	77.3	“ “ “	25	64	113.7	82.8
	27	88.8	“ “ “	28	80.7	103.7	90.9
	29.3	86.3	“ “ “	25	76	85.3	88.1
					Av.	102.4	94.5
					m.v.	9	10.9
	24	70.8	5 kg. 7½ min.	21	42.9	87.5	60.6
	20.4	83.3	5 “ 10 “	25	88	122.5	105.6
	21.1	81.9	5 “ 10 “	25	88	113.1	107.4
	17	82.3	5 “ 15 “	21	76.2	123.5	92.6
					Av.	111.5	91.6
					m.v.	12.2	15.5
	28.6	89.5	9 laps, 165 sec.	26	80.8	91	90.3
	28.7	87.3	9 “ 167 “	26.1	83.9	99.4	96.1
	27.6	85.5	9 “ 166 “	25	84	90.6	98.2
	28.4	86.1	9 “ 165 “	24.8	77.5	87.3	90
					Av.	92.6	93.7
					m.v.	3.9	3.5
	29	82.7	18 laps, 360 sec.	26	84.6	89.6	102.3
	29	85.4	18 “ 359 “	26.4	84.7	91	99.2
	30	90	18 “ 360 “	28	85.7	93.3	95.2
	30.2	88.2	18 “ 360 “	26.9	86.6	89.1	98.4
					Av.	90.8	98.8
					m.v.	1.4	2
	28	93.1	27 laps, 570 sec.	29	89.7	103.6	96.3
	28	89.3	27 “ 566 “	30.5	90.1	108.9	100.6
	27.9	88.5	27 “ 568 “	29.6	86.6	100.6	97.8
	29.4	88.3	27 “ 565 “	29.3	90.6	99.7	102.6
					Av.	103.2	99.3
					m.v.	3.1	2.3

TABLE II*a*.—MULTIPLICATION TESTS. Average number of problems, and per cent correct, from total daily records given in five-minute periods. (For number of cases, see Table II*b*.)

Subj.	Interval.	Test.	First 5 min.		Second 5 min.		Third 5 min.		Total.	
			No.	% Cor.	No.	% Cor.	No.	% Cor.	No.	%Cor.
Co.	Rest 20 min	1	7.9	57.1	7.5	65.1	7.2	69.4	22.9	68.2
		2	7.5	47.7	8.5	66.6	8.5	73.3	24.1	59.4
	Thumb 20 min	1	11.1	76.3	11.1	64.3	9.9	63.4	32.1	67.4
		2	11.7	68.7	11.4	66	11.1	68.9	34.8	68.6
	Body 20 min	1	11.8	70.5	11.5	49	11.1	58.6	34.5	61.3
		2	12.2	63	10.8	69.4	12.2	47.2	34.7	61.4
De.	Rest 20 min	1	15	75.8	14.9	84.8	15.2	74.3	45.2	78.8
		2	15.1	77.5	14.4	57.1	14.7	66.2	54.1	67.2
	Thumb 20 min	1	17.3	84.1	17.4	84.6	16.1	77	50.9	82.1
		2	17.4	80.9	17.4	84.3	17.3	70.8	52.1	78.6
	Body 20 min	1	15.9	73.6	16.2	84.4	16.6	84.9	48.3	80.9
		2	16.5	73.7	16.9	80.3	17	81.6	51.2	78.1
Ba.	Rest 15 min	1	5.6	66.7	5.4	63.4	5.6	72	16.7	70.7
		2	4.8	54.2	5.3	60.1	4.4	62.5	14.6	58.5
	18 laps, 347 sec	1	5.5	56.2	6.4	74.7	4.6	69.2	16.5	63.4
		2	5.1	54.6	4.5	45.3	4.8	71.6	14.4	57.1
	27 laps, 572 sec	1	5.3	54.2	5.2	43.3	5.8	73	16.3	57.1
		2	5.8	62.6	5.6	71.6	5.9	53.7	17.4	61.3
	36 laps, 819 sec	1	5.1	81	5.2	69	4.5	24.7	14.8	58.2
		2	4.9	90.4	4.5	82.1	4.8	60	14.2	77.5
	1½ mi. 610 sec	1	6.9	78.2	6.3	72.2	5.7	62.8	18.9	72.1
		2	6.9	79.7	6.2	66.5	5.7	68.2	19.8	72.5

TABLE II*b*.—MULTIPLICATION TESTS. Total results from each day's record, and the per cent of increase or decrease in the second test. The first test is considered 100%.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	No.	% Cor.	Interval.	No.	% Cor.	No.	% Cor.
Co.	21.4	57.9	Rest 20 min..........	23.4	55	109.3	94.9
	21	76.2	" " "	24.5	36.7	116.6	48.2
	22.7	60.3	" " "	21.4	71.9	94.3	119.2
	26.5	48.5	" " "	27	74.1	101.9	152.8
					Av.	105.5	103.8
					m.v.	4.9	32.2
	30.8	70.8	Thumb 20 min........	31	74.2	100.3	104.8
	27.5	56.4	" " "	30	76.6	109.1	118.1
	34.3	64.1	" " "	39.4	56.9	114.9	88.7
	35.8	78.2	" " "	39	66.6	108.9	85.2
					Av.	108.3	99.2
					m.v.	4	12.2
	29.7	60.6	Body 20 min..........	29	51.7	94.3	85.3
	32.7	63.2	" " "	31	67.7	94.9	107.1
	37.6	58.5	" " "	38	60.5	101.1	103.4
	38	63.1	" " "	41	65.8	107.9	104.3
					Av.	99.5	100
					m.v.	4.7	8.6
De.	37	89.2	Rest 20 min..........	40	52.5	108.1	58.9
	45	71.1	" " "	46	69.5	102.2	97.9
	52.6	82.8	" " "	47	65.9	89.3	79.6
	46.2	72	" " "	43.5	81.1	94.1	112.7
					Av.	98.4	87.5
					m.v.	6.9	18
	51	90.2	Thumb 20 min........	44	84.1	86.5	93.2
	48.5	78.5	" " "	51	74.5	105.1	94.9
	54.5	77.9	" " "	54	77.7	97.4	99.7
	49.5	81.8	" " "	59.3	78.1	119.8	95.5
					Av.	102.2	95.8
					m.v.	10.2	1.9
	42	85.7	Body 20 min..........	44	81.8	104.7	96.6
	47.6	76.9	" " "	54	74	113.4	99.1
	53.7	83.2	" " "	52.7	73.4	98.1	100.1
	50	78	" " "	54	83.3	108	106.9
					Av.	106.1	100.7
					m.v.	3.5	3.1
Do.	17	64.7	Rest 18 min..........	16.5	54.5	97.1	84.2
	22.3	55.1	" 15 "	24	75	107.6	136.1
	25.3	64.4	" " "	26.3	73.3	103.9	113.8
	21.3	57.7	" " "	23	73.9	107.9	128.1
	25.5	74.5	" " "	26	80.8	101.9	108.4
					Av.	103.7	115.1
					m.v.	3.3	14.6
	25	56	5 kg. 10 min..........	27.5	81.8	110	144.6
	25	76	5 " 10 "	24	87.5	96	115.1
	19.5	64.1	5 " 11 "	20.3	55.6	104.1	86.7
	23.3	61.3	5 " 10 "	24.5	75.5	105.1	123.1
	20.3	70.4	5 " 10 "	26.3	64.8	129.5	92
					Av.	108.9	112.3
					m.v.	8.6	18.4

TABLE II*b*—CONCLUDED.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	No.	% Cor.	Interval.	No.	% Cor.	No.	% Cor
	23.3	61.3	5 kg. 20 min	24	58.3	103	95.1
	21.5	76.7	5 “ 20 “	26	76.9	120.9	100.1
	26.5	79.2	5 “ 20 “	26.5	56.6	100	71.4
	22.3	87.4	5 “ 20 “	24.3	75.3	108.9	86.1
	24.5	83.6	5 “ 20 “	26	57.7	106.1	69
					Av.	107.8	84.3
					m.v.	6.4	9.3
Ba.	14	42.9	Rest 15 min	16.4	57.3	117.1	133.6
	14.1	78.7	“ “ “	13.4	59.7	95	74.6
	19.5	79.4	“ “ “	12.7	39.3	65.1	49.5
	16.4	67	“ “ “	12.1	57.8	73.7	86.3
	17.7	83	“ “ “	15.2	59.2	85.9	71.3
	18.5	73.5	“ “ “	18	77.7	97.3	105.7
					Av.	89	86.8
					m.v.	14.1	22
	15.1	59.6	18 laps, 347 sec	14.5	62	96	104
	18	72.7	18 “ 345 “	15.2	40.7	84.4	55.5
	16.3	70	18 “ 348 “	13.5	62	82.8	88.5
					Av.	87.7	82.8
					m.v.	5.5	17.9
	15.3	28.1	27 laps, 572 sec	13.5	40.7	88.2	141.2
	17.3	63.5	27 “ 574 “	20	70	115.3	110.2
	16.3	79.8	27 “ 571 “	18.6	73.2	114.1	91.7
					Av.	105.9	114.3
					m.v.	11.8	14.5
	14.1	64	36 laps, 819 sec	13.8	73.5	90.8	114.9
	13.8	57.2	36 “ 818 “	12.7	100	92	174.8
	16	62.5	36 “ 820 “	16	62.5	100	100
	15.4	54.2	36 “ 819 “	14.6	84.6	94.8	156.1
					Av.	94.4	136.5
					m.v.	2.7	29
	16.3	75.4	1½ miles, 610 sec	16.3	61.4	100	81.4
	19.7	45.6	1½ “ 540 “	17.1	76	86.8	166.6
	22.6	76.8	1½ “ 710 “	20	65	88.5	84.6
	19	78.9	1½ “ 710 “	19.3	79.2	101.5	100.4
	19	94.7	1½ “ 620 “	20.4	85.3	107.4	90.1
	18.2	61.5	1½ “ 660 “	20.5	68.3	112.6	111
					Av.	97.8	105.7
					m.v.	8.4	22.1

TABLE III*a*.—SOUNDER TESTS. Averages from total daily records

Subj.	Interval.	Test	First four minutes.					Second four minutes.				
			Sounds	Omissions	Errors	% of Omissions	% of Errors	Sounds	Omissions	Errors	% of Omissions	% of Errors
Bt.	Rest 20 min	1	61.3	26.3	15.5	42.5	25.7	61	22.2	10.8	35.9	17.6
		2	69.5	13.5	7.8	22	18.4	52.7	16	10.1	27.5	19.6
	Harness 20 min	1	63.8	28.4	15.4	44.4	24.4	61.2	25	11.4	40.2	18.2
		2	62	20.8	8.4	34.3	13.8	58.8	17	9.6	28.8	16.5
De.	Rest 20 min	1	62.7	21	7.6	33.4	11.8	60.1	15.7	7.3	25.2	12.1
		2	60.8	15.4	6.1	26.7	9.8	56.6	12.8	4.6	27.5	8
	Harness 20 min	1	58.4	16.6	8.4	28.2	14.6	56.7	15.7	11.7	17.9	21
		2	57.4	13	7.1	22.6	12.7	22.6	12.7	6.7	13.4	10.2
W.	Rest 20 min	1	60.3	22.6	15.5	37.5	25.7	60.3	20.8	12.2	34.5	20.2
		2	59.5	16.6	8	27.9	13.4	59.7	16.9	12.4	28.3	20.8
	Harness 20 min	1	58.8	19.6	13.2	33.3	22.5	59.7	19.8	13.9	33.2	23.3
		2	60	15.1	8.1	25.2	13.5	56.9	16.2	12.6	28.5	22.1
Be.	Rest 20 min	1	61	9	18.6	14.7	30.5	59.2	11.4	17.4	19.2	29.2
		2	62	5	6	8.1	9.7	60.4	6.4	9.2	10.6	15.2
	Harness 20 min	1	63.4	6.2	15.8	9.8	24.9	61.8	6.8	9.6	11	15.5
		2	63	6.4	8.2	10.1	13	60.2	7.4	12.4	12.3	20.6
Bu.	Rest 20 min	1	62.8	5.8	17	9.2	27.1	63.4	7	19.8	11	31.2
		2	62.6	6.6	13	10.5	20.8	59.6	5.4	10.6	9.1	17.7
	Harness 20 min	1	62.6	6	13.2	9.6	21.1	58.6	4	13.4	6.8	22.9
		2	62.2	7.8	11.4	12.5	18.3	58.8	4.8	9.2	8.3	15.6
Do.	Rest 20 min	1	63.7	15.5	19.5	24.3	30.6	64.5	18	22	27.9	34.1
		2	58.5	11.7	12.5	20	21.4	61.5	6.7	12.5	10.9	20.3
	Harness 20 min	1	60.2	9.5	16.7	15.8	27.7	62	14	19.5	22.6	31.4
		2	64	14.5	25.2	22.7	39.3	60.5	16.7	23.2	27.6	38.3
	Rest 2 hours	1	72.7	7.5	7.2	10.3	10	81.2	13.2	18.5	16.3	22.8
		2	81.7	12.2	16.2	15	19.8	85	13.2	16.2	15.6	19.1
	Walk 2-3 hours	1	68	7.2	11.7	10.7	17.3	72	14.7	15.7	20.5	21.9
		2	71	10.2	12	14.4	16.9	76.5	17.7	28.7	23.2	37.6
S.	Rest 2 hours	1	59.8	18.8	22	31.1	36.8	58.8	16.4	18.6	27.9	31.6
		2	58.2	6.8	15	11.7	22.3	60.8	10	15.2	16.5	24.9
	Walk 2-3 hours	1	57.2	12.2	17.8	21.3	31.1	57.2	12.8	12.8	22.4	22.4
		2	60.6	13.3	15.6	21.8	25.7	60.4	20.2	20	33.4	33.2

given in four-minute periods. (For number of cases, see Table III*b*.

Third four minutes.					Fourth four minutes.					Total.				
Sounds	Omissions	Errors	% of Omissions	% of Errors	Sounds	Omissions	Errors	% of Omissions	% of Errors	Sounds	Omissions	Errors	% of Omissions	% of Errors
60	24.1	15.5	34.6	25.7	59	19.8	12.7	33.4	19.1	241.3	92.4	54.5	38.3	22.6
57.1	19.1	11.1	31.6	19.3	59.8	15	7.5	25.1	12.6	239.1	63.5	36.5	26.6	15.3
59.8	21.4	10.4	35.9	17.4	59.8	22	9.4	35.2	18.9	244.6	96.8	46.6	39.5	19.1
59	22	10.6	37.4	18.4	59.8	18.6	9.6	28.8	18.4	239.6	78.4	38.2	32.7	15.9
56.6	15.3	5.7	26 3	10	61.3	14	3.1	22.1	10.3	240.8	66	25.7	27.4	10.6
53.6	13.8	4	25	7.3	58	14.1	7	24.6	12.7	229.2	56.1	21.7	24.4	9 5
57.7	12.8	4.7	21.8	8	58	9.8	4	16.7	8.7	230.8	54.9	28.8	23.8	12.5
57.7	14.3	7.8	23.9	13.6	58.5	12.5	3.7	21.2	6.4	230.3	53.2	24.6	23.1	10.7
58.1	21.7	15.2	37.4	26.2	60.8	19.5	9.5	32.1	15.6	239.5	84.6	52.4	35.3	21.9
61.4	19.1	11.7	31.1	19.1	60 7	20.1	13.2	33.1	21.8	241.3	72.7	45.3	30.1	18.9
62	17.6	12.2	28.4	19.7	56.2	17.9	11.4	31.9	20.3	236.7	74.9	50.7	31.6	21.4
59	19	10.7	32.2	18.1	57.1	19.7	11.7	34.5	20.5	233	70	43.1	30	18.5
62.2	12.4	17.8	19.9	28.6	57.4	6.8	13	11.8	22.6	239.8	39.6	66.8	12.3	27.9
58.4	5.6	8.2	9.6	14	60.6	4.6	7.4	7.5	12.2	241.4	21.6	30.8	8.9	12.7
61.2	8.8	14	14.4	22.8	61.8	4 6	9.4	7.4	15.2	248.2	26.4	48.8	10.6	19 7
60.2	8	10.2	13.1	16.9	60.4	10.1	17.4	16.7	28.8	243.8	31.9	48.2	13.1	19.7
60.6	4.2	12.2	6.9	20.1	60.4	6.4	10.2	10.6	16.8	247.2	23.4	59.2	9.5	23.9
60	4.6	10.6	7.7	17.6	59.8	4.8	10.6	8	17.7	242	21.4	44.8	8.8	18 5
60	6.2	19.4	10.3	32.3	58.6	5.4	13	9.2	22.2	239.8	21.6	59	9	24.6
59.6	7.6	13.4	12.7	22.5	60	12	15.2	20	25.3	240.6	32.2	49.2	13.4	20.4
63.5	16.5	22.5	25.9	35.4	62.5	14.5	20.7	23.2	33.1	254.2	64.5	84.7	25.4	33.3
60.5	9.2	14	15.2	23.1	61	18.5	25.2	30.3	41.3	241.5	46.1	64.2	19.1	26.6
59.7	13.2	15.2	22.1	25.4	63.5	16.2	27.2	25.5	42.8	245.4	52.9	78.6	21.1	32
61.5	12.2	21.2	19.8	34.5	61.5	10.2	19	16.7	31.4	247.5	53.6	88.6	21.7	35.8
82	13.5	15.7	16.5	19.2	81.5	20.2	20.7	24.8	25.5	317.4	54.4	62.1	17.1	19.5
87.2	18	17	20.6	19.5	92.2	15.7	14.5	17	15.4	346.1	59.1	63.9	17.1	18.4
74.2	14.7	18	19 9	24.2	72.2	14.5	17 2	20.1	23.9	286.4	51.1	62.6	17 8	21.9
77	7 5	15.2	9.7	19.8	86.2	19	24.7	22	28.7	310.7	54.4	80.6	17.5	25.9
57	18.6	15.2	32.6	23.2	63	19.8	19.8	31.5	31.4	238.6	73.6	75.6	30.8	31.7
59.4	14.6	14.2	21.2	23.9	55.4	14	15 8	25.3	28.5	233.8	45.4	60.2	18.9	25.7
57.8	15.8	11	27.3	19	59.2	14.6	10.8	24.7	18.2	231.4	55.4	52.4	23.9	22.6
59.8	14.6	15.4	24.4	22.4	59.4	17.4	15.6	29.3	22.6	240.2	65.4	66.6	27.2	27.7

TABLE III*b*.—SOUNDER TESTS. The per cent of total omissions and errors from daily records, and the per cent of increase or decrease in the second test. The first test is considered 100%.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	% O.	% E.		% O.	% E.	% O.	% E.
Bt.	38.4	38.9	Rest 20 min	37.8	39.6	98.4	101.8
	29.9	20.1	“ “ “	18.4	6.6	61.5	32.8
	54.5	33.2	“ “ “	27.2	14.3	49.9	43.1
	38.6	14.9	“ “ “	36.5	12.3	94.5	82.5
	33.7	11.5	“ “ “	31.9	15.7	94.6	136.5
	26.9	13	“ “ “	14.5	8.1	53.9	62.3
					Av.	74.6	73.5
					m.v.	20	30.4
	24.9	15.4	Harness* 20 min	23.8	18	95.5	116.8
	50.4	28.6	“ “ “	45	16.4	89.2	57.3
	43.5	19.6	“ “ “	31.9	11.4	73.3	58.1
	42.5	22.8	“ “ “	35.7	16.1	84	72.8
	35.2	12.7	“ “ “	32.9	17.6	93.4	138.5
					Av.	86.2	80.8
					m.v.	6.9	29.6
De.	12.9	7.6	Rest 20 min	9	7.6	69.7	68.4
	51.6	17.4	“ “ “	43.7	16.7	84.6	95.9
	39.2	10.2	“ “ “	26.9	6.4	68.6	62.7
	30.1	10.8	“ “ “	29.3	15.5	97.3	143.5
	12	3.4	“ “ “	18.7	5.5	115.8	161.7
	21.2	9.1	“ “ “	16.9	44.9	79.7	54.9
	20.8	15.4	“ “ “	24.2	11.2	116.8	72.7
					Av.	89.5	87.7
					m.v.	16.7	32.8
	27.3	16.4	Harness 20 min	7.4	5.6	42.7	34.1
	31.5	19.6	“ “ “	28.1	18.3	89.2	93.3
	31.2	9.6	“ “ “	33.9	13.3	108.6	138.5
	29.5	18.9	“ “ “	40.2	13.8	136.6	73
	14.8	7.1	“ “ “	10.7	4.3	72.3	60.5
	14.7	6.9	“ “ “	15.5	7.5	105.4	108.7
	27.1	8.5	“ “ “	28.8	11.2	106.2	131.7
					Av.	99.1	85.1
					m.v.	26.2	31.3
W.	31.7	24.2	Rest 20 min	36.1	20.3	113.8	83.8
	23.7	23.9	“ “ “	27.4	15.6	119.1	65.2
	53.7	34.1	“ “ “	34.4	25.8	64	75.6
	47.6	24.4	“ “ “	51.7	37.1	108.6	152
	37.1	16.7	“ “ “	37	12.6	99.7	75.4
	30.8	16.2	“ “ “	24.7	17.4	80.1	107.4
	36.4	26.7	“ “ “	29.5	17.1	81	64
	33.7	16.6	“ “ “	14.6	5.9	43.3	35.5
	28.9	26.9	“ “ “	21.4	15.4	74	57.2
	28.6	9.8	“ “ “	26.5	9.8	76.5	100
					Av.	82.1	80.5
					m.v.	18.7	22.1

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* Lifting body ergograph with harness.

TABLE III*b*—Continued.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	% O.	% E.		% O.	% E.	% O.	% E.
W.	34.9	20.9	Harness 20 min.......	29.1	18.4	83.3	88
	26.7	24.5	“ “ “	20.8	18.2	77.9	74.6
	17.3	15	“ “ “	22	16.7	127.1	111.3
	51.1	32.4	“ “ “	44.4	16.1	86.8	49.7
	31.4	20.1	“ “ “	31.6	17.3	100.6	86
	42.6	26.6	“ “ “	39.4	18.7	92.4	70.3
	32.9	17.7	“ “ “	31.5	19.9	95.7	112.4
	24.4	14.8	“ “ “	26.3	9.6	107.7	64.8
	35.8	14.1	“ “ “	29.1	17.1	81.2	128.3
	20.3	15.2	“ “ “	23.9	13.4	117.7	88.1
					Av.	94	82.1
					m.v.	12.7	19.1
Be.	11.8	31.3	Rest 20 min..........	12.3	18.1	104.2	57.8
	26.4	38	“ “ “	13.8	12.5	52.3	32.9
	14.9	21.1	“ “ “	10.1	13.2	67.8	62.5
	9.6	13.1	“ “ “	4.3	8.6	44.7	65.6
	19.7	35.1	“ “ “	4	10	20.3	28.5
					Av.	57.9	49.5
					m.v.	20.5	13
	10.4	18.8	Body 20 min.........	16.8	26.4	161.5	140.4
	7.9	20.2	“ “ “	13.9	23.7	175.9	117.6
	10.5	17.2	“ “ “	9.1	17.4	86.6	101.2
	15.5	27.4	“ “ “	18.1	24.7	116.8	90.1
	9.2	14.6	“ “ “	7.6	6.8	82.6	46.4
					Av.	124.7	99.1
					m.v.	35.2	24.7
Bu.	4.9	29.8	Rest 20 min..........	8	21.5	163.3	72.1
	7.3	29.3	“ “ “	12.3	24.1	141.1	82.2
	6.1	9.6	“ “ “	.4	2.9	6.6	30.2
	23.7	43.1	“ “ “	20.7	34.8	87.3	80.7
	4.7	6.9	“ “ “	3	6.9	43.5	100
					Av.	88.4	73.2
					m.v.	51.1	17.5
	10	13.9	Harness 20 min.......	14.7	11.8	114	84.9
	5.9	47.1	“ “ “	8.6	18.2	145.7	59.7
	2.4	14.5	“ “ “	.8	7.2	33.3	49.6
	16.2	35.2	“ “ “	24.9	40.1	153.7	113.9
	10.7	11.5	“ “ “	17.6	12.6	164.5	109.6
					Av.	128.4	83.5
					m.v.	38.7	23.1
Do.	37.5	38.6	Rest 20 min..........	21.6	30.3	57.6	78.4
	7.7	8.1	“ “ “	9.9	6.3	128.5	77.7
	23.6	35.6	“ “ “	15.7	21.8	66.5	61.2
	32.9	51.2	“ “ “	30.3	50	91.8	97.6
					Av.	86.1	78.7
					m.v.	23.8	6.9
	20.6	25.7	Harness 20 min.......	18.8	28.5	91.2	110.9
	23.8	31.3	“ “ “	24.4	47.6	106.7	152.1
	29.3	56.6	“ “ “	18.4	42.9	62.8	75.8
	11.5	17.9	“ “ “	15	20.3	130.4	113.4
					Av.	97.8	113.1
					m.v.	20.9	19.7

TABLE III*b*—CONCLUDED.

	1st Test.			2d Test.		% of Inc. or Dec.	
Subj.	% O.	% E.		% O.	% E.	% O.	% E.
Do.	13.1	14.3	Rest 2 hours	16.6	14.8	126.7	103.5
	15.6	17.2	“ “ “	9.8	22.7	62.8	131.9
	26.9	31.3	“ “ “	30.4	23.1	113	73.4
	11.7	10.7	“ “ “	7.6	17.4	64.9	162.6
					Av.	95.8	105.9
					m.v.	28	29.4
	13.8	38.4	Walk 2 hours	13.1	29.1	94.9	75.7
	17.2	25	“ 2 “	17.9	30.3	104	120.1
	31.5	25.4	“ 2¼ “	34.9	39.5	110.7	159.8
	9	4.3	“ 3 “	6.2	7.4	68.9	172
					Av.	100.5	114.1
					m.v.	12.7	37
S.	34	35.1	Rest 2 hours	22.2	36.7	65.3	104.5
	28	23.2	“ “ “	13.6	8	48.6	34.5
	28.8	34.5	“ “ “	26	32	90.2	92.7
	33.9	34.9	“ “ “	14.5	20.9	42.7	59.8
	26.4	26.9	“ “ “	16.1	27	60.9	100.3
					Av.	61.2	87.8
					m.v.	12.9	23.1
	27.7	20.5	Walk 2 hours	21.4	22.7	77.2	110.7
	23.8	21.7	“ 2 “	36.6	33.6	153.3	154.4
	20.7	14.5	“ 2½ “	21.9	19	105.3	131
	16.9	20.9	“ 2¼ “	28.8	31.8	170.4	152.1
	34.7	39.2	“ 2 “	27.7	23.8	79.8	60.7
					Av.	110.5	111.9
					m.v.	32.5	31

Table IV*a*.—Association Tests. The per cent correct and the average time of the daily records, and the per cent of increase or decrease when the learning followed physical fatigue. The corresponding records when learning followed rest are considered 100%.

		After Rest.		After Harness.		% of Inc. or Dec.	
Subj.		% Cor.	Av. Time.	% Cor.	Av. Time.	% Cor.	Av. Time.
R.		82	10893	47.6	3588	58	39.2
		78.6	6772	67.9	8237	86.4	121.6
		60.7	4977	50	6308	82.3	126.8
		67.9	5757	35.7	13004	52.6	224.1
		82.1	7741	35.7	4234	43.4	54.7
		60.7	5000	64.3	11857	105.9	237.1
		71.4	4584	28.6	14001	40.1	305.4
	Av.	71.9	6532	47.1	8747	66.9	157.5
	m.v.	7.7	1660	11.8	3606	21.1	81.1
M.		71.4	1485	57.1	4024	79.9	270.9
		92.9	2305	89.3	3766	96.1	163.3
		82.1	3476	60.7	3488	72.7	100.3
		82.1	1897	71.4	3317	86.9	174.9
		96.4	3717	92.9	3328	96.4	89.5
	Av.	84.8	2576	74.3	3585	86.4	159.8
	m.v.	7.7	816	13.5	258	8.1	51.9
Br.		55	7039	40	7026	72.7	99.8
		70	7691	65	9008	92.8	117.1
		72	10399	60	8161	83.3	78.4
		75	6712	61	6597	81.3	98.3
		62.5	6595	45	10141	72	153.9
	Av.	66.9	7687	54.2	9986	79.4	109.5
	m.v.	6.5	1086	9.4	1861	6.7	50.8
				(After Running 2 Miles.)			
E.		35	3500	70	5520	200	157.7
		32.2	5726	75	7692	232.9	134.3
		65	6175	40	3819	61.5	61.8
		25	6829	20	1314	80	19.2
	Av.	39.3	5557	51.2	4588	143.6	93.2
	m.v.	12.8	1029	21.2	2019	72.8	52.7
				(After Walking 2½ Hours.)			
H.		20	2824	25	7463	125	260.7
		40	2940	37.5	14012	93.7	476.6
		35	5412	83.3	6997	237.1	129.3
		55	5047	72.2	4315	131.3	85.5
		45	4624	44.4	5633	98.6	121.8
	Av.	39	4169	52.1	7326	137.1	214.8
	m.v.	9.2	1030	20.1	2751	39.9	123.1
Do.		40	3453	15	7519	37.5	217.7
		70	4609	50	3093	71.4	67.1
		40	8267	45	6315	112.5	76.4
		81.2	5973	25	5505	30.8	92.1
		40	9282	45	6311	112.5	67.9
	Av.	53.5	6292	32.5	5645	66.2	99.9
	m.v.	14.7	1680	14.1	1068	33	39.3

TABLE IV*b*.—ASSOCIATION TESTS. The average association times taken from the total individual results, and the distribution of extremely long and short times.

Subj.	R.	M.	Br.	E.	H.	Do.
After rest. Per cent correct,	71.9	80.7	67.	47.	40.6	54.4
Average time	5931	2765	7426	4706	4449	5966
No. of cases	74	59	34	15	24	34
No. > 10,000σ	13	2	5	2	3	8
No. < 3,000σ	23	41	5	4	7	11
After fatigue. Per cent correct,	47.1	70.2	54.2	51.2	50.9	35.4
Average time	9488	3159	6151	5595	7172	5442
No. of cases	42	52	30	22	35	23
No. > 10,000σ	9	2	7	4	6	4
No. < 3,000σ	7	32	8	13	8	11

DISCUSSION OF RESULTS.

The consideration of results that deal in any way with physical fatigue is certain to raise the question as to how much physical fatigue, general or special, has been produced in a given individual by the method used. This it must be confessed is a difficult question to answer in any case, and particularly so in the present set of experiments where mental tests were to be given which must not be disturbed more than is necessary. For this reason, and also because of the difficulty of analyzing the chemical products of fatigue, the attempt to determine the physical fatigue by measuring these products was abandoned. Furthermore, it is doubtful whether a very definite evaluation of the degree of fatigue could be secured in this way. The only remaining possibility seemed to be to test the physical capacity before and after the work period, and compare the results with those obtained before and after an equal rest interval. For this purpose a large dynamometer was used. This instrument was similar to the ordinary hand dynamometer, only much larger, and was fastened to the floor by means of a chain attached to one end of the spring. A wooden handle was attached to the other end of the spring. The subject exerted himself in as nearly as possible the same position as was required at the beginning of the lift with the wall machine. The result was disappointing. The subject, either because he shifted his position slightly, or because for a single pull he exerted more effort, was able to exert as much or more force on the dynamometer after he had worked at the wall machine. For example, Ca. was able to lift 100 kilograms before the work period. At the end of seven and one-half minutes of lifting a weight of 5½ kilograms he was forced to stop from exhaustion. Yet he then lifted 110 kilograms. This method was, therefore, abandoned, and the general habits and physical development of each subject were used as a basis upon which to approximate the value of a given amount of work for the production of fatigue in each individual.

The form of the ergographic curves obtained with the two spring ergographs may, to some extent, indicate the degree of fatigue, though these curves do not, as a rule, resemble very closely the form of such curves as we find them in the literature. There was always a quite rapid fall in the height of the curve during the first three minutes, and by the end of five minutes it had reached a level that was generally maintained until the end. The principal exceptions were the curves of W., which decreased rapidly during the first three minutes and then more and more gradually to the end. At the end of the twenty minutes his curves were still decreasing. Whether the knowledge that the work was expected to endure for twenty minutes compelled the subjects unconsciously to conserve their strength, or whether the realization that the curve was falling acted as an incentive (the curves were not screened from the subject) to exert more effort, as Wright (47) found, or whether the employment of a large group of muscles allowed sufficient shifting of the burden to alter the form of the curve, our work furnishes no data to determine. It is quite probable that more than one of these factors had an influence. Both ergographs gave the same form of curves and both were open to these possibilities, though the possibility of shifting with the thumb ergograph was not so great as with the lifting ergograph.

ADDITION TEST.

With Ca. speed in adding has always been increased after rest and decreased two out of three times after physical work. Two factors might influence accuracy: an increase (or decrease) in speed might mean a corresponding decrease (or increase) in attention to accuracy of the processes; or it might mean greater or less mental ability expressed in terms of accuracy of the association processes independently of the association time. The subjects were always instructed to add as rapidly as was consistent with accuracy, and it was expected that attention would be about equally divided between speed and accuracy. The emphasis was not to be placed on either phase of the test to the exclusion of the other. We might, therefore, assume that this sort of variation would be slight, except as it was brought about by the fatigue. We are left with the conclusion that for Ca. physical fatigue tended to decrease speed and to increase accuracy. That Ca. was physically fatigued was quite certain. As has been mentioned, he was not a physically strong man, and he worked faithfully.

Similar fluctuations are evidenced in the results of the other two subjects. Da. nearly always showed increased efficiency in the second test, whether it is after rest or physical work. His greatest increase is in speed after rest, but this is partially counterbalanced by a decrease in accuracy. With an increase in the duration of the physical work or an increase in the weight to be lifted his speed decreases and accuracy increases, very much as in the case of Ca. On the other hand, Ba. is inclined to show a decrease in both speed and accuracy after both rest and physical work, though this is not always the case. After lifting a light weight ten minutes speed is increased, while accuracy is about the same as after rest. The nine-lap and eighteen-lap runs are quite uniformly followed by decreases in speed and accuracy, on the whole, a little below the decreases manifested after the rest periods; but the twenty-seven-lap runs effect an increase in speed, and an increase in accuracy half the time, though the average of the four tests gives a decrease. While these differences for Ba. are slight, they are quite constant. Certainly the weight of five kilograms could not be considered very fatiguing for Da. and Ba. With the heavier weight Da. seemed fatigued, though not what could be considered exhausted. Running on the indoor track was only slightly fatiguing for Ba. at the end of nine laps; he "felt tired" at the end of eighteen laps (one mile) ; and he thought twenty-seven laps was the limit of his endurance. Beyond thirty-six laps (in the next series) he would not attempt to run.

MULTIPLICATION TEST.

Ba. was the only subject to be used with both the addition and the multiplication tests. His results, therefore, form the connecting link between the two tests for a basis of comparison. If we examine Tables I*b* and II*b*, we find that after rest he shows a decidedly greater decrease in both speed and accuracy with the multiplication tests than with the addition test. As multiplication is a much more difficult process than addition as the two were employed, we may assume that the mental fatigue induced by the first test has not entirely disappeared at the end of the twenty-minute rest interval, or that his interest is not so great. It is true that the five-minute periods of the first tests, as given in Table II*a*, do no indicate any accumulation of fatigue, but this may be obscured by practice effects or overcome by inertia effects. The details of tables I*a*, II*a* and III*a* (pp. 213-222) will be discussed later. After eighteen laps he does better than after rest, but not so much better as in the addition test. After twenty-seven and thirty-six laps he shows marked improvement, though after thirty-six laps speed (on the average) has given way somewhat to an unusually large increase in accuracy. A large part of this variation is due to the test following the thirty-six laps on the second day of the series, though the general tendency for decreased speed and increased accuracy is seen throughout the series.

Just where in the total series the tests with the cross-country running should be considered to belong is difficult to determine. These runs were made over hills and uneven paths, usually in a strong wind. Ba. believed he was more fatigued than when he ran two miles (thirty-six laps) on the track, but other runners claimed that they always found it less fatiguing than the same distance indoors, providing they ran more slowly across country. Ba. in this series required considerably more time than he did indoors for the same distance. It was expected that the series would be completed by a series of five-mile runs across country, but a change in Ba.'s plans made it necessary to abandon this plan. It is necessary, therefore, to consider this series separately. The results would indicate that the fatigue was a little more than that produced by eighteen laps indoors.

In the tests with Co. and De. we have a different set of conditions. The time spent in physical work by these subjects was the same in each (twenty minutes), but the amount of physical fatigue produced was controlled by the type of work performed. A much smaller, isolated group of muscles was used with the thumb ergograph than with the larger apparatus. It might be expected that if physical fatigue affects mental ability by virtue of the circulation of fatigue products from the working muscle, that the effect of the thumb ergograph would not be so great.

The influence of these two types of work is not very clear in the results obtained. The differences in speed and accuracy are not very great or constant. De. increases speed after rest twice and decreases it an equal number of times, but decreases accuracy three out of four times. In the two work series he has increased speed a little on the average, and increased accuracy slightly after the thumb exercise. Variations from day to day lead to little hope that any influence of physical fatigue can be recognized in his results. Even the big average increase in speed after rest is made in spite of two decreases out of four tests. An average of the per cent of increase or decrease in both speed and accuracy computed from Table II*b* gives 102.5 per cent after rest, 99 per cent after thumb exercise, and 101.2 per cent after body exercise. Neither De. nor Co. show the decrease in mental ability after twenty minutes' rest that is indicated by Ba.'s results. Co. has consistently increased speed after the thumb exercise, though accuracy is increased but twice and decreased an equal amount the other two times. He has not been able to produce an average increase after the body exercise, though he was as liable to increase as to decrease.

Just how fatigued either De. or Co. might have been would be difficult to estimate. Both were normally developed men, but they certainly found the work difficult. It is believed that each worked conscientiously. If he did so, he must have been greatly fatigued, in the arm when using the thumb ergograph, and more generally in the legs and shoulders when using the larger apparatus.

The results from Do. do not vary materially in speed. This may be due to the method employed. It will be remembered that he conducted the tests alone, and used a stop watch to determine the fifteen-minute period. The problems were also mimeographed on letter-size paper, in place of on numerous narrow slips as in the other experiments. Both of these variations when taken together might influence the subject's speed. Though the number of problems performed in a period were not counted until the entire series was finished, a certain amount of space on the sheet might represent to the subject a certain amount of work done. This knowledge, coupled with the awareness that a certain amount of time had elapsed, might spur the subject to greater activity in case he were falling behind his usual amount. As the accuracy of the work was not determined until the entire series was finished, no more knowledge was possible in this case than the other subjects possessed regarding their accuracy.

Accuracy is distinctly improved in every instance after rest except in the first test. The recorded introspections show that the subject felt greatly fatigued from the first test of the first day, which the eighteen minutes of rest did not seem to relieve. This may account for the discrepancy of the first day's results. The lack of knowledge of the relation of the space covered to the amount of work done should also be considered a factor in this discrepancy. After lifting five kilograms ten minutes accuracy is improved nearly as much as after rest, but the variations are greater. After twenty minutes' lifting there is a notable decrease in accuracy with a relatively small variation from the average.

As was stated earlier, Do. could not have lifted the weight for twenty minutes at the beginning of the series. At least he thought he could not. The ten-minute periods seemed decidedly fatiguing even in the last test, though they never compared with the twenty-minute periods, which were extremely exhausting.

SOUNDER TEST.

This test differs from the addition and multiplication tests in an important respect. Speed as a variable factor in the subject's work is eliminated by the sounders being operated at a regular rate, which the subject must follow. This results in an incentive for the subject to keep sufficiently alert to discriminate the sounds as they are given in order to prevent absolute confusion. The accuracy of the discrimination alone, rather than the speed and accuracy of the association processes, is all that is measured. The speed of the subject's discrimination and reaction may be recorded by this method for a short series, but for a period of sixteen minutes it would be impracticable, as it would necessitate running the kymograph rapidly in order to record distinctly small units of time.

As the kymograph was run, the horizontal distances between the marks that indicated the sounders and the subject's reaction were only about one-fourth of an inch. Consequently little could be determined as to the subject's reaction time. A very long paper would be necessary in order to measure accurately the effect of fatigue upon the time of reaction to sounders.

It will be noticed that there is nearly always a decided increase in ability to discrinimate the sounds in the test following the rest periods for all subjects except for Do. in the case of the two-hour rests. The sounds were frequently slightly different on one day from what they were the previous day, but the principal factor seemed to be that the subjects seemed to forget the sounds from one day to the next, and the first test served as a means of relearning the relation of the four sounds. The results of Do. would bear out this assumption. As he operated the apparatus for all the other subjects, he was kept more familiar with the sounds the year he was subject with the two-hour rest periods, and consequently did not show improvement in the second test. During the year that Do. acted as subject with the twenty-minute rest periods the sounders were placed in one room with the subject and the rest of the apparatus was in an adjoining room. This prevented the experimenter from hearing more than a very slight click when he operated the sounders, which was in no way similar to the click of the sounders when heard in the same room with them. It will be observed that Do.'s results in this series conform to those of the other subjects.

Though improvement was manifested after work by three subjects, it was not nearly so great as after the rest interval. It may be that we have here, with the exception of the work of Do., the influence of physical fatigue not only upon the ability to discriminate the sounds, but also upon the memory process. This might mean that the learning process in the first test has been interfered with in some way by the physical work that follows, or that the recall in the second test has been affected by the physical fatigue. Mr. De Camp has conducted a series of experiments (as yet unpublished)* to determine the influence upon learning nonsense syllables of different kinds of work following at various intervals after the learning. The interval between learning and recall was usually only twenty minutes, and it might be possible in these experiments for fatigue to affect recall as well as to exert a retroactive effect upon learning. However, a part of the series were tested twenty-four hours after learning. In these, as well as in the series tested twenty minutes after learning, he found very little evidence of a retroactive influence when mental work was used. In the tests in which physical work was used after learning the tests showed a lower per cent of recall, but, as the physical work was always used in the short intervals, it is probable that it affected the results through the influence of physical fatigue on the recall, rather than that it affected the learning process. This is Mr. De Camp's interpretation of the results. If we accept his conclusions as applicable to our sounder test, then we find that physical fatigue has affected our test either by decreasing the power of discrimination or by interfering with the practice effects (recall) of the first test.

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* Since the above was written Mr. De Camp's work has appeared in Psychological Monographs, vol. 19, No. 4.

That interference with the practice effects is not the only influence of fatigue is demonstrated by the results of Do. Though after rest there was an average of only 4.2 per cent decrease in omissions, which was counterbalanced by 5.9 per cent increase in errors, he shows an average increase of 0.5 per cent in omissions and 14.1 per cent increase in errors after walking for two hours. Though S. improved greatly after rest, he shows as decided decrease in discrimination after the physical fatigue. Inasmuch as fatigue could influence the practice effects only to the point of rendering no improvement, these results must be due, in part, at least, to the direct influence of fatigue upon attention and discrimination.

The introspections of the subjects throw further light upon the nature of the processes with which the sounder test deals. In every case it was reported that the sound to be reacted to was held in mind and the other three sounds were dropped out. Confusion always resulted when the other sounds were not inhibited. In this the results and introspections uniformly agreed. To test the degree to which this inhibition process was effective, an extra minute was occasionally added to the second test without the knowledge of the subject, and only two sounders used, the one to which the subject was to react, and one other. In no case did the subject know what had occurred, though he usually realized near the end of the minute that some change had taken place. It never materially disturbed his reactions. Be. reported that the three sounds were kept in mind, but were not clearly distinguished, that is, each sound had a number for her, and she knew whether it had been given as the reaction sounder, but otherwise the three were confused. For Do. the extra three sounds were generally scarcely more clearly present than any other slight noise that might occur, and when one was signaled as the sound to be discriminated, it came as a sound that had not been heard during the previous minute. Only one subject, a student whose results were too meager to be reported, found that he kept all four sounds clearly in mind. If he failed to do so he became confused.

The two types of physical work used with the sounder test should be considered separately. The "harness" represents a strenuous type of work that generates fatigue quickly. The subject is somewhat out of breath, feels weak in the knees, and is more or less in a tremor all over. The long walks develop fatigue much more slowly, and there is more time for a gradual readjustment generally. Moreover, physical fatigue produced by walking has more time, if time is needed, in which to affect mental ability. Two subjects walked, and one of them was used in the work with the harness. So there is a slight basis furnished for a comparison of the two kinds of physical fatigue. After the walks S. shows an unusual decrease in power to react correctly; this condition is fairly constant throughout the series. In only one test has he shown improvement in both the per cent of omissions and in the per cent of errors; in one other case he has improved in omissions but failed in errors. Usually a change in one form of mistake is paralleled by a similar change in the other form. This is not true with Do.'s results. The two forms are more inclined to fluctuate independently. His failure after walking is also not so marked as in S.'s results. It will be noticed that the per cent of mistakes in both tests for Do. on the last fatigue day is extremely low. It is difficult to account for this unusual drop. It was not the fault of the sounders, for S. was tested at the same hour, and his results are normal. The sounders were operated a little faster for Do. than was usual, and it may be that this acted as a stimulus to greater activity, or the fact that the sounds came closer together may have made them easier to discriminate. Possibly this is merely an indication of unusual mental activity. If this was true in regard to his mental condition it certainly was not true in regard to his physical condition in the second test. Do. had walked three hours as rapidly as possible and had covered fifteen miles. Nausea accompanied his feelings of fatigue.

It is quite certain that the other subjects were considerably fatigued by the harness exercise, particularly W., who worked most conscientiously and whose curve fell gradually throughout the entire twenty minutes. More tests were taken with W. than with any other subject, but his results vary between wide limits. There seems to be no constant tendency in his results in the relation of omissions to errors; they neither vary together nor oppose each other. It will be noticed that the fourth rest day produced an unusually large increase in the per cent of errors and a considerable increase in the per cent of omissions. The eighth day reversed this and gave extremely large decreases in both omissions and errors. On the whole, however, the tendency is to decrease the improvement after physical work and the fluctuations are not so great. Do. shows a slightly greater decrease in the per cent of errors after the physical work than after rest, but the per cent of omissions has increased to a much greater extent after work than the omissions have decreased. Bt. shows a decided decrease in improvement after work and his daily records are fairly constant.

Be. manifests the greatest influence of physical fatigue. Her improvement after rest is uniformly great, and after fatigue she almost as uniformly falls below her record in the first test. Bu. is very irregular. It will be noticed that he frequently makes very few mistakes, and at other times makes as many as the other subjects. It is impossible to account for this extreme variability, as Bu. was always a conscientious, intelligent worker, and the tests were always conducted under as nearly the same conditions as possible. He was our youngest subject and of only sophomore rank. It is possible that his variability was due to lack of training.

A comparison of Do.'s results with the harness and with the walking indicates that the harness has reduced discrimination power as much as walking. If we consider the improvement after twenty minutes' rest, then the harness has had the greater effect. It is unfortunate that we have two sets of conditions here, as it makes it uncertain whether the effect is due entirely to the mode of producing physical fatigue or to the possibilities of learning in the tests. However, it is certain that both modes of physical fatigue have decreased mental efficiency in a marked degree. All the subjects have shown some decrease in mental ability after physical work as tested with the sounders. This decrease has been greatest for Be. and S., and moderately large for Bt. The other subjects, except Bu., have been affected definitely. Bu.'s results are uncertain.

ASSOCIATION TEST.

As in the sounder test, any variation in speed that can affect in any way the accuracy of the association processes has been eliminated. The variations in the time of recall on the following day represent the degree to which the syllables had been learned, and do not indicate any possible changes due to the influence of fatigue upon the process of recall. The syllables were exposed at a regular rate after rest and fatigue periods and the only possible effect of the physical fatigue would be upon the learning process. The effect of fatigue upon the process of recall constitutes a distinct problem that should be studied, but the present series deals only with the influence upon the learning process as evidenced by recall after a period that is long enough that fatigue can not affect recall directly.

The per cent correct and the average association time for each day's test are given in Table IV*a*. As the fatigue days alternated with the rest days, the results for corresponding days are placed opposite each other in the first four columns. The per cents of increase or decrease for the fatigue days as compared with the results for the rest days are given in the fifth and sixth columns. The total per cent correct, and the average time, taken from the total scores, without reference to the individual days, are given in Table IV*b*, which also shows the number of cases used in determining the average time, and the number of cases in which the time was more than 10,000 σ and the number of cases in which it was less than 3000 σ. It should be remembered that the total number of cases given represents those responses that were correct or partially correct. The incorrect responses were not used in determining the average association times.

Four of the subjects have shown decreased learning power after the physical work. Of these, three not only have made lower scores but have required longer times for the recall. The fourth, Do., has a slightly shorter average time of recall after physical work, which seems to be due to the elimination of a few of the extremely long times. The average times after work for the other three subjects is due to a general increase in the time of recall. On the other hand, H. has a better score after work, but his average time is much greater, due in part to more extremely long times, but also due to a more general lengthening of the time. The results of E. are very doubtful. Two of the days following rest he has a higher per cent correct than on the corresponding work days, but the association time has been longer for these days. His average time is longer on work days in spite of a much greater number of extremely short times. Were it not for the fact that his averages here are very similar to results of his work in other experiments, to be reported in another paper, we might consider these results as of little value. The indications of these later experiments are that in a longer series of association experiments with physical fatigue the average result would have been approximately the same as it is here. Furthermore, we can not consider him as thoroughly fatigued after running two miles across country as most of the subjects were after their form of physical work, as E. was a regular track man in training at the time. H., who presents a consistently higher average after the long walks, was accustomed to long walks every day, and usually reported only slight feelings of fatigue. It is significant, also, that there is a general tendency with most subjects to make a greater effort to recall when learning followed fatigue. At least this is suggested by the generally longer association times and more extremely long times.

The results of R. deserve separate consideration, as they do not involve purely rote learning as the others do. Whereas all cases involving sensible associations were discarded in the other series, R. was allowed to form all the associations he desired. Nevertheless, his results were quite as consistent as those of the other subjects. In only one case has he made a better score after physical work than on the corresponding rest day, and then the association time is unusually long. Only two work days produced higher scores than the poorest rest day. That the mental processes involved in learning a series on nonsense syllables as R. did are different from those employed in the rote learning by the other subjects, can not be doubted. At least they are more complex. The following are a few of the syllables learned and the associations by which they were connected:

bex hup; *bec*kons to hurry *up*.
gam cib; *gam*ble, punish at *Sib*eria.
roc gub; *roc*ks, money for *gub*ernatorial campaign.
wip cej; *wip*e, *see j*ust a little better.
huv cef; *hu*rry *v*ery fast to *see Eph.*

These are samples of the connections he formed. All the other syllables were connected by similar associations. Undoubtedly physical fatigue influenced these associations materially, as he shows the greatest effect of physical fatigue and more frequently reported difficulty in forming connections after work periods than after rest periods.

The results of M. are very uniform, with the exception of the scores for the first rest day and the first work day. There is nothing to indicate why these scores should be so low, as the practice series that preceded usually gave better averages. If we omit these two scores, the total averages would be 88.4 per cent after rest and 73.4 after work; that is, a little greater decrease in learning after work would be manifested. Br. and Do. show considerable tendency to fluctuate from day to day, but their scores are consistently lower on work days.

THE CURVE OF MENTAL WORK.

The addition and multiplication tests were divided into five-minute periods, and the sounder test into four-minute periods, in order to determine, if possible, the effects of inertia and fatigue within the course of the mental work of the test. The addition and multiplication tests could have been divided into smaller periods, but the variation in the difficulty of individual problems made the longer periods seem advisable. The time required to solve each problem was always recorded by the experimenter, and several curves were constructed by one-minute periods and by individual problems. These curves were so irregular and varied so widely from each other for the same individual that the plan was abandoned and the longer periods adopted.

The sounder test could not have been divided into smaller equal periods, for the four sounders presented unequal difficulties for discrimination.

The chief characteristic that is brought out by the subdivision of the addition and multiplication tests is the almost total lack of any constant tendency, either in the speed or accuracy of the processes. In Table I*a* one may find instances in the first tests that indicate that Ba. decreases speed after the first period and increases accuracy, but there are other instances in which he shows no such tendency. This is illustrated by the tests before rest and before running eighteen laps. In the second tests these series show the reverse arrangement. Co. (Table II*a*) manifests a constant speed in all averages of tests preceding the interval, and a fairly constant speed following the interval, whether it is after rest or physical work; but there is no uniformity in accuracy in either the first or second tests. Even the subjects that did more poorly in the second tests on work days do not show definite signs of fatigue in the first tests. In the results with the sounder tests (Table III*a*) there is a more definite tendency observable for some subjects. Bt. improves throughout the first tests, though his average in the second quarter is better than in the third in the series before rest. In the second tests his accuracy decreases until the fourth period. De. improves regularly in the first tests and in the first three quarters after rest, but the fourth quarter is lower than the first. After fatigue he decreases gradually in efficiency until the fourth quarter and then manifests a decided improvement. This might be due to a realization of the fact that he had been doing poorly and that the end must be near. All the subjects realized approximately the progress of the multiplication test. The other subjects, however, showed less definite tendencies, except that W. always did better in the first quarter of the second test and gradually decreased in the following quarters. It seems probable, therefore, that if such phenomena as the "antrieb" and the "anregung" do exist, they are, as Thorndike (39) finds them, purely occasional occurrences which lack any great importance. In general, a single test may be selected from any subject's series that illustrates any theory desired, but the average of several tests represents a blur. As was indicated earlier, the mean variation, if given, would be very large.

CONCLUSIONS.

One of the common fallacies in the study of fatigue, Squires (36) has pointed out, is to consider it as a simple phenomenon. It is true we may isolate a muscle and produce a fatigue that is relatively simple, but in the usual conditions of what we term fatigue we are dealing with a complexity that is difficult to analyze. If we accept Thorndike's definition of fatigue as "that diminution of efficiency which rest will cure" the problem seems simplified. But we still have to reckon with the physiological and mental factors that bring about this diminution of efficiency, and their relative influence is not always so easily determined.

We find that the effect of physical fatigue upon mental efficiency, as expressed by addition and multiplication, is very irregular. A subject may do better after a work period than after rest, or he may show an increase in speed and a decrease in accuracy. This agrees with the results of Ebbinghaus (10) and those of Heck (15) in their experiments on mental fatigue in school children. Again, the subject may improve after physical work in proportion to the amount of work done; thus, Ba. failed after rest, did less poorly after running eighteen laps, and improved after twenty-seven and thirty-six laps. The sounder and association tests, on the other hand, present more uniform results, and, in most cases, show a diminished efficiency after physical work.

Two theories present themselves as explanations of these results. (1) It may be that the fatigue products generated by the physical work, either in the motor neurones or in the muscles, spread through the circulation to the association centers and cause directly a diminution in the efficiency of the association processes; or, (2) it may be that the sensations that accompany fatigue serve as distractions and thus cause a decreased attention. The former view is partially borne out by the results. As in Ranke's (33) experiments the perfusion of an isolated muscle with a weak solution of sarcolactic acid caused a heightened excitability, so it is possible that a certain amount of physical work would produce a sufficient amount of fatigue substance to cause increased mental activity; a greater amount of this fatigue substance would cause a decreased mental activity. Ba.'s results (Table II*b*) would then indicate that the increased physical fatigue caused by running the longer distance had transmitted to the association centers only relatively small amounts of fatigue products, though the motor system might contain much greater concentrations. We might, therefore, expect that he would show greater mental fatigue later, or after a greater degree of physical fatigue, than was reached in these experiments. It is doubtful, however, if this theory could be made to account for all the facts.

That physical fatigue may influence mental efficiency by virtue of its distraction power is evidenced by many little incidents during the course of the experiments and by the introspections of the subjects. In all but the association tests it was possible for the experimenter to mark the record during the course of the test whenever he wished to record any change in the conditions of the experiment. These records could then be checked with the report of the subject at the end of the test. It was found that what the subject reported to be a distraction affected his results in just the same way that it has been found that fatigue may do. Sometimes it caused a decrease in mental efficiency and at other times it caused an increase. Moreover, the results did not always agree with what the subject thought the effect of the disturbance had been. In general, a slight noise, such as the peeping of some chicks that were in the room at one time, or a person talking outside near the door, disturbed the subject, but increased his efficiency. On the other hand, louder noises, or longer continued disturbances, were apt to be accompanied by a decrease in efficiency.[*]

It is highly probable that physical fatigue operated as a distraction in much the same way. The accompanying sensation of physical fatigue and the general condition of the subjects after physical work may be gathered from their introspections. The following are representatives: "Felt shaky in legs, but not sleepy. Mind seemed clear." "Back and feet ached. Did not feel fatigued until about twenty minutes after I stopped physical work." "Could not keep results (multiplication) in mind. Arms seemed rigid at the end of the work period; then grew weak and numb." "Shoulders felt sore. General tension all over. Cobwebs seemed cleared away in second test." "Mind seemed clear at the beginning of second test, then felt a tendency to confusion. Felt drowsy in second half of the test. Legs tingled and hips ached." "Mind clear at first; then a tendency to sleep. Muscles of whole body seemed to be relaxing. Were tense at first." "Felt sleepy all over, *i. e.*, numb and quivery, particularly in legs. Could read the syllables easily, and mind seemed fairly clear, but the syllables seemed to make little impression." It will be seen that frequently the subject feels stirred up and active after the physical work. Or he may start the second test in that condition and then he notices the coming on of drowsiness and the general relaxation of the muscles. Sensations of strain have given way to sensations of relaxation or of rest, which seems to constitute the feeling of fatigue.

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[*] Since the above was written a series of experiments to study the influence on the sounder test of various forms of distraction has been undertaken by Miss Berger in the Kansas laboratory. So far as the work has progressed the results seem to conform closely to the above casual observations.

Shepard (35) suggests that "the content of sleep consists of a group of sensations of 'fatigue' or 'rest.' Sleep is a more complete rest. The process is a dominance of an organized group of these sensations. Such sensations from one part are associated with those from another. It is not that the sensations are aroused only at the time of sleep, of course, but that they become dominant in attention as any other group of sensations may be dominant in attention. This dominance is promoted by the intensity of the sensations themselves and by other conditions of attention." (p. 78.) This relation of the sensations of fatigue or relaxation to the mental condition is seen in the introspections. One subject reported that the arms seemed rigid, and so long as this condition of strain endured he was mentally alert. At least he experienced no tendency to drowsiness. We simply can not sleep when the sensations of strain dominate. But as the muscles begin to relax, drowsiness and consequent confusion result.

There remains the difference in the character of the results with the first two tests as compared with the last two tests to be considered. This difference can be accounted for by the effect of incentive. It is doubtful whether we ever do our best unless there is present something more than the vague purpose to do our best. A runner can run a little faster in a contest than he can alone, however hard he may try. Wright (47) found that a subject could do considerably more on the ergograph when he was given a standard or incentive than he could do when he was merely told to do his best all the time. In the addition and multiplication tests there was no standard, or at best only a vague one, but in the sounder and in the association tests the subjects had to keep up to the standard of speed set by the experimenter, and he must keep mentally alert all the time in order to avoid complete confusion of the sounders. The result was less possibility of variation, and consequently less chance to obscure the effects of physical fatigue.

Though the addition and the multiplication tests are valuable tests, the sounder and association tests furnish the more reliable data in this series. The association test was thought to involve more complex processes, and it was expected originally that it would yield more readily to the effects of physical fatigue. However, the results do not differ materially. Further studies on the effect of physical and mental fatigue upon the attention and association processes are now in progress, and will be reported later.

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A Study of Germ Cells of *Corymorpha palma.*

LUCIE M. MARCH.

Pates LI-LIII.

(Contribution from the Zoölogical Laboratory, No. 217.)

INTRODUCTION.

THE problem which is here described was undertaken at the suggestion of Dr. Bennet M. Allen, to whom I am indebted for assistance and guidance throughout the entire extent of the work. Originally, the problem was to discover the origin and path of migration of the germ cells of *Corymorpha,* but, as observations accumulated, it has enlarged itself to include the formation and development of the medusa, particularly the formation of that part called by Weismann the "glockenkern," which has not been worked out in any detail before. While the problem of the origin is not completely settled here, the germ cells have been traced to a point beyond which they can not be followed, owing to the limitations of the material at hand and the methods of technique.

LITERATURE.

A vast amount of work has been done upon the germ cells of cœlenterates. An excellent review of the results and interpretations of various workers has been given by Hegner in his recent book on "The Germ-Cell Cycle in animals," so it will be unnecessary to devote any space here to the discussion of the general problem.

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While much has been done with the germ cells of other hydroids, comparatively few workers have taken up *Corymorpha.* Weismann observed only a single female medusa of *Corymorpha,* but describes this form in his "Die Entstehung der Sexualzellen bei den Hydromedusen" (1883), and gives a drawing to show ova in the ectoderm of the manubrium. He finds the older ova in the manubrium, and describes germ cells similar to the young ova among the ectodermal cells in the neck of the manubrium. He finds the entoderm to contain no cells that could be called germ cells, and concludes that the germ cells arise in the ectoderm. Although he observed no male medusa, he draws the conclusion that the male germ cells must arise in the same way as the female cells in *Corymorpha,* since they do in other allied forms.

May[*] has described the morphology and development of *Corymorpha pendula,* the eastern species, which differs from *Corymorpha palma,* the western species, in only a few minor points. He describes the general morphology of the form, and takes up the development of the medusa. He finds the medusa buds beginning as simple evaginations of the wall of the peduncle. "By a proliferation of ectodermal cells at the distal end of the bud, a plug of ectodermal cells is formed, which grows down into the medusoid cavity forcing back the entodermal cells as it advances." He states that these cells of ectodermal origin give rise to the future reproductive elements, but fails to show how and when definite germ cells arise, and does not trace them through the early stages of development. As the ova develop, certain cells are said to develop at the expense of others, by a process of absorption through pseudopodia-like processes and by the breaking down of cell boundaries, forming a syncytium of cells in which disintegrating nuclei may be seen.

Investigations by Torrey[†] indicate a third process, the incorporation of cells which have previously begun to disintegrate. Torrey, in speaking of *Corymorpha palma,* says the germ cells arise in the ectoderm of the manubrium from cells which have been derived from the ectodermal plug, at the apex of the medusa bud. Torrey's other papers on *Corymorpha** fail to throw any additional light upon the subject of the origin or migration of the germ cells.

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[*] May, Albert J. 1903. A Contribution to the Morphology and Development of *Corymorpha pendula.* The American Naturalist, Vol. 37, No. 441.

[†] Torrey, Harry Beal. 1907. Biological Studies on *Corymorpha.* II, Development of *C. palma* from the Egg. University of California Publications. Zoölogy, vol. 3, No. 12.

MATERIAL AND TECHNIQUE.

The material from which these observations were made was collected at Pacific Beach, Calif., during the summer of 1914. Some of the material was fixed in Flemming's fixative, some in Telleyesniczky's fluid, and some in Zenker's. All of these fixatives gave good results, but, on the whole, the Flemming material was most satisfactory.

At first single medusæ were cut longitudinally and transversely, and an attempt was made to isolate and section in a definite plane small pieces of the peduncle, bearing one or two of the younger buds, in hopes of getting all stages of development, but it has proven more satisfactory to use the whole peduncle with all but the largest medusæ attached. By this method there is always the chance of cutting a few of the buds in the proper plane, and the sex of the older medusæ serves as a guide to the sex of the younger buds, since medusæ of only one sex develop on a single peduncle.

The sections were all cut four or five micra in thickness. Benda's Mitochondria stain, Rubaschkin's stain, Bensley's neutral gentian stain, and iron-hæmatoxylin with eosin or Congo red were used in staining the sections. Iron-hæmatoxylin with neutral gentian as a counter-stain proved the best for differentiating germ cells, and the combination of these two stains was used for most of the sections.

I.—FORMATION AND DEVELOPMENT OF THE MEDUSA.

Young medusæ of *Corymorpha palma* usually develop in groups as outpushings from the stalks of older buds, although they frequently occur singly along the main peduncle. The first indication of the formation of a new bud is a clumping of entodermal cells and a rounding out of the ectoderm in this region (figure 2). At the time of this outpushing the nematocyst cells have practically disappeared from the ectoderm of the bud, while the ectoderm and mesoglea both are noticeably thinner here than in other regions of the peduncle (figure 1). This stretching and thinning of ectoderm and mesoglea continues as development proceeds (figure 3).

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* Torrey, Harry Beal. 1902. Hydroids of the Pacific Coast of North America. University of California Publications. Zoölogy, Vol. I, No. 1.

1907. Biological Studies on *Corymorpha*. I, *C. palma* and Environment. Journal of Experimental Zoölogy, Vol. I, No. 3.

Further development of the medusa is shown in figure 3. The bud has elongated and there is a decided thickening of the ectoderm at its apex. The cells here are crowded together and the cytoplasm has rounded up around the nucleus so as to give a dense appearance to the whole region. The ectoderm of the sides and base of the bud consists of a single layer of cells. The cells of the entoderm have come to lie in a row just beneath the mesoglea, those at the tip being more definitely organized than the others farther back. A loose mass of structureless cytoplasm occupies the center of the bud, and a cavity is forming just beneath the well-organized cells at the apex.

The next stage (figure 4) shows the origin of a structure to which Weismann applied the term "glockenkern." Weismann says, "Als Entocodon oder Glockenkern bezeichne ich jenes wichtige Embryonalorgan, durch dessen Vermittlung die einfach blindsackförmige Knospe zur Meduse umgewandelt wird, jene schon von so vielen Autoren gesehene und unter verschiedenen Bezeichnungen beschriebene Wucherung des Ektoderms in der Spitze der Knospe, welche den Entodermschlauch eindrückt und so zur Bildung einer becher—oder kelch—förmigen hohlen Entoderm—Duplikatur Anlass giebt, aus welcher die Entodermlage der Medusenglocke hervorgeht. Es is nötig, dafür eine bestimmte, einfache und unzweideutige Bezeichnung zu haben."* This "glockenkern" originates from the thickened mass of ectodermal cells at the tip of the bud. The ectoderm and entoderm have separated in this region and some of the ectoderm cells have passed into the cavity formed by this separation. The "glockenkern" cell mass thus formed later gives rise to the subumbrella and the ectoderm of the manubrium, which functions as a germ gland. As this "glockenkern" cell mass enlarges it pushes back the layer of entoderm cells beneath it.

Figure 5 shows the further development of the "glockenkern." The whole bud has increased in size, and the pear-shaped cell mass has grown down into the central cavity of the bud, pushing back the endoderm as it advances. Germ cells are first seen in the "glockenkern" at this stage, and there is a tendency for the cells to become arranged in two layers, a deeper layer next to the entoderm, containing the germ cells, and an outer layer, composed only of somatic cells.

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* Weismann, August. 1883. Die Entstehung der Sexualzellen bei den Hydromedusen.

A later stage in the development of the medusa bud is shown in figure 6. There is a continued increase in size, and the "glockenkern" has spread out to form an inverted cup-shaped mass around a central elevation of entodermal cells. This central elevation indicates the general shape of the manubrium, and that part around the outside, where the entoderm turns sharply back upon itself, will form the umbrella of the medusa. The cells of the "glockenkern" have now separated into two distinct layers. The outer layer, composed only of somatic cells, lies closely pressed against the ectodermal layer surrounding the whole bud. The inner layer, composed of some somatic cells and all of the germ cells which have migrated into the "glockenkern" at this time, lies close to the entodermal cells beneath. This deeper layer is the ectoderm of the manubrium, which serves as a germ gland in *Corymorpha*. The outer layer of somatic cells becomes the lining of the umbrella.

As the medusa bud develops, the split between the two layers of cells in the "glockenkern" completely separates the manubrium from the outer capsule of the bud (figure 7). At the tip of the bud this outer capsule is composed of only two layers of cells. It is at this point that the developing manubrium later breaks through the surrounding capsule. Around the sides of the manubrium, in the region of the umbrella, the capsule is composed of four layers of cells, two layers of entoderm formed by one continuous layer folded back upon itself, an outer layer of ectoderm, and an inner layer of ectoderm which has been derived from the "glockenkern" cell mass.

The cavity of the manubrium and the cavity of the umbrella are both continuations of the central cavity of the bud, which, in turn, communicates with the cavity of the peduncle. A cross-section of a bud in this stage, taken through the manubrium and the umbrella (figure 8), reveals the fact that the cavity of the umbrella is not uniform, but shows clearly the formation of the four radial canals. The germ gland rounds out to form four ridges down the sides of the manubrium, which press together the entoderm in the corresponding regions of the umbrella. In the intermediate regions between the ridges of the manubrium the entodermal cell layers are widely separated, forming the four radial canals.

As the medusa bud grows, the manubrium increases in size until it finally breaks through the outer capsule at the tip, where there are but two layers of cells (figure 9). Further development of the bud involves no radical change in the arrangement of cell layers. The tips of the radial canals widen out and meet to form the circular canal. The apex of the manubrium never opens to the exterior. The sexes are separate, and the germ cells, either eggs or sperm, are developed in the ectoderm of the manubrium, and shed, when ripe, while the medusa is still attached.

In the fully developed medusæ there is a distinct difference between the sexes. The male medusa has a relatively longer manubrium and shorter umbrella than the female medusa, and the manubrium of the male is more blunt at the tip than that of the female.

Sexual differentiation is not apparent until fairly late stages of development, but the sex of even the very earliest buds may be determined by their relation to the older medusæ in which the sex is evident. Since medusæ of only one sex are found on a single peduncle, the sex of young buds will always correspond to that of the older medusæ borne upon the same peduncle.

II.—THE ORIGIN, MIGRATION AND DEVELOPMENT OF THE GERM CELLS.

1.—*Distinguishing characters of early germ cells.* The earliest germ cells to be found in *Corymorpha palma* are marked by certain characters which serve to distinguish them from other cells, entodermal or ectodermal. The nucleus of a primitive germ cell, wherever it may be found, is usually larger than the nuclei of other cells, but this difference in size does not always serve as a means of identification. The nucleolus is large and prominent. The nucleus is much less dense than the surrounding cytoplasm, and the deeply staining chromatin is condensed into coarse masses which lie around the periphery, close to the nuclear wall. The combination of these two characters, the clearness of the nucleus and the arrangement of the chromatin, gives to the nucleus a hollow, rounded appearance which is absolutely distinctive and serves as the safest means of identification. The cytoplasm of the primitive germ cell is usually more dense than that of other cells, and contains coarse, deeply staining granules, probably mitochondria. The limits of the primitive germ cells are usually quite definite, marking off the germ cells from other cells among which they are found.

2.—*Origin of the germ cells.* Primitive germ cells appear scattered throughout all parts of the ectoderm of the peduncle, but are most numerous near the bases of older medusæ in regions where new buds are developing (figure 1).

3.—*Migration of the germ cells.* In order to follow the path of the migrating germ cells it has been necessary to arbitrarily establish certain stages in the development of the medusa bud. These stages are based entirely upon differences in structure. Since all of the material used for these observations was fixed material, there is absolutely no way of measuring the length of time involved in the development of the successive stages.

Stage A includes all buds from the merest outpushing up to stage B (figure 2).

Stage B includes only the elongated buds with a distinct thickening of the ectoderm at the apex (figure 3).

Stage C includes those buds having the undifferentiated "glockenkern" cell mass between the ectoderm and entoderm at the tip of the bud (figure 4).

Stage D includes all buds having the pear-shaped "glockenkern" (figure 5).

Stage E includes buds similar to the one shown in figure 6.

Primitive germ cells are found scattered throughout the ectoderm of the peduncle, but are not seen among the ordinary entoderm cells. In all cases where there is any indication of the formation of a medusa bud, and in all later stages, germ cells are found among the ectoderm cells of the young bud. Whether the presence of germ cells serves as a stimulus for the development of the bud, or whether the development of the bud attracts the germ cells, can not be determined, but the presence of germ cells in the entoderm of the earliest buds can not be denied. The fact that germ cells are normally present in the ectoderm, and are not present in the entoderm, except in the region of developing buds, is a strong indication of migration from ectoderm to entoderm.

In counting the germ cells in each bud, not only the number but the position of the cells has been taken into consideration in order to determine the migration. Some of the larger buds in stage A and all of the buds in later stages were divided into three parts, proximal, central and distal, and the germ cells were counted in the entoderm and ectoderm of each of these regions. The average of at least two counts was taken as the final number for each bud.

TABLE I.

		Entoderm.			Total	Ectoderm.			Total	
Bud.	Sex.	Prox.	Cent.	Dist.	entoderm.	Prox.	Cent.	Dist.	ectoderm.	Total.
1	?	..	..	..	16	..	..	..	8	24
2	?	..	..	..	12	..	..	..	9	21
3	?	..	..	..	14	..	..	..	7	21
4	Male,	..	..	..	9	..	..	..	6	15
5	Male,	4	9	4	17	10	4	1	15	32
6	Male,	4	7	1	12	7	2	..	9	21
7	Male,	5	9	1	15	3	2	..	5	20

Table I shows the number of germ cells in stage A medusa buds. Buds 1, 2, 3 and 4 were the youngest in which germ cells were counted, and were too small to divide into different regions, but the presence of a considerable number of germ cells in the entoderm at this time and the presence of others in the ectoderm of the peduncle at the sides of the buds indicates the early migration of the germ cells toward the bud. The relative number of germ cells in the different regions of the older buds, 5, 6, and 7, presents a fairly good indication of the path of migration followed by the germ cells. The predominance of germ cells in the proximal third of the ectoderm and the predominance of germ cells in the central portion of the entoderm seems to show the passage of such cells from ectoderm to entoderm at a point somewhere within the limits of these two regions. The path of migration, then, is probably from ectoderm to entoderm at the base of the bud, and then among the entoderm cells toward the apex. The germ cells in the distal part of the ectoderm are probably abnormal in position, since there are so few, and since there is no indication of a continued migration in that direction.

Allowance must be made for individual variation in the matter of migration. Some medusæ show an early and a rapid migration of germ cells, while others show the process delayed. There is apparently no constant difference between the sexes as to this question of migration, nor with regard to the number of primitive germ cells in the early stages.

TABLE II.—Number of Germ Cells in Stage B Buds.

Bud.	Sex.	Entoderm.			Total entoderm.	Ectoderm.	Total.
		Prox.	Cent.	Distal.			
1	Male	7	17	4	28	1	29
2	?	4	8	5	17	5	22
3	Male	12	9	1	22	7	29
4	Female	7	8	2	17	6	23

Table II gives the number of germ cells in stage B. In this stage there is an increase in the number of germ cells in the entoderm, accompanied by a corresponding decrease in the number of germ cells in the ectoderm of the bud, without any decided change in the total number as compared with the total number in the previous stage.

TABLE III.—Number of Germ Cells in Stage C Buds.

Bud.	Sex.	Entoderm.			Total entoderm.	Ectoderm.	Total.
		Prox.	Cent.	Distal.			
1	?	7	14	..	21	1	22
2	?	3	7	5	15	2	17
3	Female	4	7	3	14	4	18
4	Male	5	11	..	16	3	19
5	Male	8	11	1	20	6	26

Table III shows the number of germ cells in the medusæ at stage C, the time of the formation of the "glockenkern." There is no great change in the total number of germ cells, but the arrangement is somewhat different. The reduction in the number of germ cells in the ectoderm shows that they are still passing from ectoderm to entoderm, without being replaced by other germ cells from the adjacent ectoderm of the peduncle. The continued predominance of germ cells in the central third of the entoderm, and in increase in the number in the distal third, indicates a constant migration toward the "glockenkern" at the apex.

TABLE IV.—Number of Germ Cells in Stage D Buds.

Bud.	Sex.	Entoderm.			Total entoderm.	Ectoderm.	"Glockenkern."	Total.
		Prox.	Cent.	Dist.				
1	?	6	11	1	18	3	4	25
2	Female	2	3	3	8	..	11	19
3	Female	8	13	3	24	1	..	25
4	Female	3	10	7	20	2	..	22
5	Female	3	7	3	13	1	7	21

In stage D (table IV) some germ cells have passed into the "glockenkern," and the germ cells have practically disappeared from the ectoderm, without any marked change in the total number. The fact that in the two buds, 3 and 4, no germ cells have migrated into the "glockenkern" probably indicates nothing more than an individual variation in the form of a delayed migration.

TABLE V.—Number of Germ Cells in Stage E Buds.

		Entoderm.			Total		"Glocken-	
Bud.	Sex.	Prox.	Cent.	Dist.	entoderm.	Ectoderm.	kern."	Total.
1	Male	..	..	3	3	..	25	28
2	Female	..	1	2	3	2	22	27
3	Male	..	1	3	4	..	19	23
4	Male	..	..	..	..	..	32	32
5	Male	..	1	..	1	..	25	26
6	Male	..	2	7	9	2	13	24

In stage E, shown in Table V, a large majority of the germ cells, and in some cases practically all of the germ cells, have passed into the "glockenkern." The germ cells still in the entoderm are in a position to migrate later into the "glockenkern" and will probably do so.

4.—*Multiplication of germ cells and sexual differentiation.* Throughout the development of the medusæ thus far there has been no apparent increase in the total number of germ cells, and no visible sexual differentiation. After stage A there is a multiplication of germ cells in the "glockenkern" and a differentiation of the cells into eggs and sperm in the different medusæ.

The egg cells increase enormously in size, the cytoplasm becomes somewhat vacuolated and yolk granules are deposited in it. There is no indication of the actual engulfment of one cell by another, nor the absorption of disintegrating cells by others (figure 10).

In older male medusæ, the number of sperm is considerably greater than the number of eggs in the manubrium of female medusæ of corresponding size. The complete process of spermatogenesis can be observed in a single bud (figure 11). The germ cells are rapidly dividing in the region near the entoderm, and later stages are seen near the periphery. The process of differentiation results in a condensation of cytoplasm and nucleus, forming an almost spherical dark body, the sperm. No attempt was made to follow the details of this process.

III.—SUMMARY AND CONCLUSIONS.

1. The medusa bud begins development as an out-pushing of the wall of the peduncle, usually near the base of the older medusæ.

2. The "glockenkern" arises as a mass of ectodermal cells pushed into a cavity at the apex of the bud, formed by the separation of the ectoderm and entoderm.

3. The "glockenkern" cell mass separates into two cup-shaped layers, one going to form the inner lining of the umbrella, the other, containing germ cells, going to form the ectoderm of the manubrium.

4. As these two layers of the "glockenkern" cell mass push back the entoderm, the manubrium becomes separated from the outer capsule (umbrella) of the bud.

5. The manubrium breaks through the outer capsule, leaving the umbrella free.

6. Primitive germ cells, identified by certain definite characters, are found scattered among the ectodermal cells of the peduncle, most frequently near the base of older medusæ.

7. The presence of germ cells in the entoderm only in the region of developing buds, indicates the early migration of some germ cells from ectoderm to entoderm at that point.

8. The path of migration taken by the germ cells is from ectoderm to entoderm at the base of developing buds, then among the entoderm cells and into the "glockenkern" at the apex of the bud. The entrance of the germ cells into the "glockenkern" does not take place until after the latter is well formed.

9. The number of primitive germ cells entering into the formation of a single medusa is fairly constant, varying from 17 to 32 in all the medusæ counted.

10. Multiplication of germ cells and sexual differentiation does not occur until a late stage of development.

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CONTENTS:

HISTOLOGY OF *Malva rotundifolia*, ... *Grace McCrone.*

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Histology of *Malva rotundifolia*.

GRACE McCRONE.

Plates LIV-LIX.

MALVA ROTUNDIFOLIA is a low-growing mallow common in this section of the country, and generally growing as scattered individuals or small groups, but sometimes occurring in large patches. It begins growth early in spring and lasts until very late in autumn.

The material used for this investigation was obtained on the University campus. The standard histological methods were employed, and the illustrations were made from permanent slides stained with safranin and hæmatoxylin and from free-hand sections otherwise treated. Microchemical tests were used on both free-hand and microtome sections. Some of the drawings were made by use of the camera lucida on the microscope and some with the projectoscope.

STEM.

The main stem branches perfusely close to the ground. The branches start up in a vertical direction, but as they become numerous the older branches lie prostrate on the ground for a greater part of their length. This position has a noticeable effect on the tissues of the stem, there being less bast fiber tissue on the lower than on the upper side, and in the starch sheath there is more starch in the side near the ground. The branches are about two or two and one-half feet long.

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The epidermis of the stem is closely covered with hairs, which are of the same general type as those of the leaf (figs. 4 and 8). The hairs are mostly stellate, with from five to nine rays. Each ray is a single cell, the center of which stains darker with safranin than the outside, giving the appearance of a core. There are also many simple unicellular hairs, similar in structure to one ray of the stellate hair, and many glandular hairs, knoblike in shape (fig. 8, *f*). These latter are frequent on the young parts of the stem and petiole and large veins of the leaf. The epidermal cells of the stem (fig. 7) are rather irregular in size and shape but have a general rectangular form. The stomata (fig. 7, *a*) vary more in shape than those of the leaf and are slightly larger. They stand with the long axis parallel with the long axis of the stem and are not so close together as those of the leaf.

Collenchyma is always present in young stems (figs. 25-26, *b* and 30-33, *a*) and in the petioles of the leaves (fig. 22, *c*), but in older parts of the stem it is thrown off when cork (figs. 34 and 35, *a*) is formed.

The vascular bundles are of the usual collateral type, and the bast fibers stand just outside of the phloëm strands, making a broken line around the outside of the phloëm (figs. 32, 33 and 38). The vascular bundles vary in size, and a large and a small one or two large ones and two or three small ones are often found alternating. The cross section of the very young stem is irregular in shape instead of being circular as in the older parts of the stem, and the large bundles are likely to be where the stem is prominent (fig. 30). In the older part the stem is nearly circular and there is less irregularity in the size of the bundles (figs. 30-35).

The changes in structure from the new to the older parts of the stem are shown in figures 30-35. The sections from which the illustrations were made were taken from the stem, first just below the growing point, second about two inches below, and at intervals all of the way down the stem to the root, representing six different regions of the stem. In the first four sections the pith covers the greater part of the diameter, but in sections nearer the base of the stem the pith becomes smaller and is nearly crowded out of existence.

The bast fibers are found in sections about three inches from the apex of the stem, a bundle of bast fibers standing before each phloëm strand. As the stem grows older the original bast fiber bundles are pushed outward and apart, and new bast fibers are produced by the cambium, until in the older parts one finds groups of bast separated by phloëm tissue and extending in radial rows (figs. 34 and 35).

The starch of the stem is all found in the starch sheath. This in the young parts of the stem forms a continuous layer of cells composed of the innermost cells of the thin-walled parenchyma of the cortex. In the older parts the bast fibers appear just inside the starch sheath. Then as the bast fiber region is extended radially the starch sheath follows these radial extensions of the bast fibers. Figures 33, *b*, to 35, *b*, illustrate this.

Wood fibers (fig. 18) are also formed and the older parts of the stem become quite woody. As the xylem develops the pith becomes smaller, the water-conducting area becomes much greater, as may be seen by a comparison of figures 30 to 35, the tracheal tubes becomes somewhat larger and their frequency is greatly increased. Figures 34-35 and 41-42 show details of water-conducting tissue.

The number of vascular bundles varies greatly, ranging from fifteen to twenty-three.

Crystals of calcium oxalate (fig. 19) occur closely packed together, sometimes in groups of cells and in other instances in isolated cells, in the pith and in the parenchyma of the cortex and pericycle. The crystals may be found any place in the parenchyma, but are more common along the bast fiber region. These crystals are all compound, and are often almost large enough to fill the cells which hold them. Figure 17, *a*, shows the distribution of crystals in the parenchyma cells of an old part of the stem.

Mucilage is found in large quantities in the stem (fig. 15). In the parenchyma of the cortex the cells containing it break down to form long tubes or passages filed with mucilage. These cells (fig. 24, *a*) sometimes also break down in lateral directions, making the mucilage cavities much larger in lateral diameter than the ordinary parenchyma cells, but in other instances the mucilage-filled cells (fig. 25) are not so much larger than the other cells. As the cells enlarge, in the older parts of the stem, the mucilage cells do not grow accordingly, and even get considerably smaller (figs. 25 and 26, *a*) as the other cell walls press against their walls. Figure 16 shows some of these cells cut obliquely, showing the great amount of mucilage in some places. In some instances these tubes measure more than one mm. in length. There are also many mucilage cells in the collenchyma, but they are not generally larger than the other cells (and therefore too small to show to the scale of figs. 27 and 28) and not many tubes are formed. The pith contains many long tubes filled with mucilage (figs. 15, 27 and 28) much the same as those in the parenchyma of the cortex.

The plant from which this study was begun was brought into the laboratory in June, and was in the second year of its growth. From observations made from this plant it was determined that there is relatively much more mucilage in the young part of the stem, that is, within two or three inches from the tip, than in the older parts. Actual counts and measurements showed this, but later, in October, more material was obtained and in this mucilage was not quite so evident in any part, and especially not near the tips of the branches.

The amount of mucilage is much less in sections near the root than near the apices of the branches, while the starch is more abundant near the root. The root contains only a trace of mucilage during the growing season, while it is well filled with starch at all times. In the autumn there is a slight increase of starch in the root and a great increase in the mucilage content (fig. 29).

Crystals of calcium oxalate are very numerous in the parts of material gathered in October, but that obtained in June showed no trace of crystals. In material collected in early spring, however, crystals were present.

The cells of the borders of the pith are relatively small and heavy-walled, while in the center of the pith the cells are much larger, the walls thinner and more regular in shape, being round or nearly hexagonal where they have pushed together and crowded out the air spaces. In the older parts mucilage is seldom found in the pith.

LEAF.

The orbicular leaves are borne on long, slender petioles and spread out almost horizontally, except in hot or dry weather when the edges curl up. They vary in size from an inch and one-half to two inches in diameter. The margin is indented to form seven shallow lobes, and through the middle of each lobe runs one large vein from which all of the veins branch to form a more or less close network of small veins.

The leaf is dorsiventral, with palisade tissue on the upper side only. The palisade cells (figs. 8, *c*, 9, *c*, 10, *a*) are usually closely packed together, with rather large air chambers beneath the stomata (fig. 10, *c* and *d*, and figs. 5 and 6) which connect with the numerous air spaces between the cells of the spongy parenchyma.

The surfaces of the leaf, both upper and lower, are covered thickly with hairs of three different kinds. The most numerous of these are stellate hairs having from five to nine rays (fig. 4). There are more hairs to the square millimeter on the young leaf than on the fully developed one. The average number of hairs on the young leaf is 36 to the square millimeter. This is just after the leaf has unfolded. A gradual decrease in number was found in leaves of successive ages, fully developed leaves having 13 to the square millimeter. Beside the stellate hairs, along the veins and along the margin of the leaf, are numerous one-celled hairs about the same in structure as one ray of the stellate hair. The surface of the hair is smooth. The center stains darker than the wall, giving the appearance of a core. The cells from which these hairs arise form a slightly raised papilla, while the stellate hairs are on a considerably raised papilla. The third kind mentioned are glandular hairs (fig. 8, *f*). These are many-celled hairs and are generally found at the veins. They are the same in structure as those found on the petiole and young parts of the stem. The nucleus in the cells of these hairs is always clearly visible.

The cells of the epidermis are very irregular in size and shape and the walls are thin (fig. 13). Waterproofing is furnished by a thin cuticle merely. Many of the epidermal cells are mucilagenous (fig. 13, *e*). Whether this mucilage is a modification of the cell wall or not has been discussed by Kuntze (1891). He describes the mucilage of the epidermis, which is characteristic of the mallow family, as being a modification of the cell wall. He quotes Dumont as believing that in some species at least the mucilage has a schizogenous origin. It is certain that young cells are sometimes stratified. In older tissue the walls appear to entirely dissolve and leave the mucilage tube or pocket. Kuntze says that it is generally the inner wall of the epidermis that swells most in water. This is true of *Malva rotundifolia* to some extent, but it is not always the case. It is stated as a characteristic of *Malvaceæ* that the epidermis of the leaves strongly swell in water, in some species all of the cells and in others only certain cells, and these sometimes in the upper epidermis only. In *M. rotundifolia* the mucilage is only in certain cells of the epidermis, and there is much more in the upper than in the lower.

Stomata are found on both sides of the leaf (fig. 18) in about the same numbers. They are more numerous in the fully developed leaf than in the young leaf. The average number to the square millimeter of the fully developed leaf is about 494, and of the young leaf 370. The stomata are in the same plane with the other epidermal cells or but very slightly project (fig. 10, *c* and *d*). There are extensive air spaces beneath the stomata, and the spaces in the spongy parenchyma communicate freely with them. Chloroplasts seem to be less numerous in the palisade cells than in the spongy parenchyma.

The veins are embedded in the spongy parenchyma, and only the main veins reach out to the epidermis. All veins are surrounded by large very thin-walled border parenchyma (fig. 14) whose cells have their long axis parallel with the length of the veins. The veins end free in a group of tracheids (figs. 1, 2, 3 and 11, *h*). Near the margin where the large veins terminate these groups are very complex (fig. 1, *a*, and 3). In each lobe of the leaf the central rib gives off lateral veins almost at right angles, in fact all of the angles in the branches of veins approach nearly a right angle. The veins are well distributed, making a close network throughout, with the ultimate branches standing about .09 millimeter apart in the old or fully developed leaf, and .11 millimeter in the young leaf.

ROOT.

The root is a tap-root going down more than two feet into the ground. There are but few lateral roots and no large ones. The bark of the root is very thick, covering about two-thirds of the diameter. Cork is formed on the outside as on the lower parts of the stem. The root contains a great amount of food material stored in form of starch, sugar, mucilage and protein. During the growing season there is only a trace of mucilage in the root, and not as much starch and sugar as in the winter season when all parenchyma cells are well filled with starch, and there are large cells and tubes of mucilage thickly scattered through the same tissue. There is also a quantity of sugar distributed through the tissues. Grape sugar is present in a considerable quantity in both the first and second year's growth. The bark is much thicker than on the stem, the cells in general are larger and the water tubes are much larger.

Botanical Laboratory,
University of Kansas.

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CONTENTS:

DIFFERENTIATION OF THE OAKS BY HISTOLOGICAL METHODS, *F. W. Mulsow.*

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Differentiation of the Oaks by Histological Methods.

F. W. MULSOW.

Plates LX-LXIV.

IN this work four species of native oaks are compared as to their histological structures and characteristics. The species studied are *Q. rubra, Q. schneckii, Q. coccinea* and *Q. macrocarpa,* as classified by Britton and Brown. Branches, leaves and acorns from one tree only of each species were studied throughout their essential parts. But in the study of *Q. rubra* and *Q. schneckii* three different trees were studied in a few of their structures peculiar to themselves, in order to find out the constancy of their characteristic structures. Parts of the leaves were embedded in paraffin and sectioned with the rotary microtome; also, some of the leaves were bleached for studying the cell structure and leaf venation. Parts of the branches were placed in dilute glycerine, this making them easier to section. The double stain of safranin and hæmatoxylin was used in differentiating the various tissues. The staining for temporary mounts may be carried out in one operation by mixing equal parts of each stain and placing the sections in this mixture for a few minutes. After washing in seventy per cent alcohol they are ready for studying in dilute glycerine. For best results the stain should be made up fresh just before using.

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THE LEAF.

The gross structure of the leaves is quite different in each of these oaks. The leaf of Q. *rubra* is somewhat smaller than either of the others, has a smooth upper surface and a dark green color. It is not so deeply lobed as the others and is less flexible. The leaf of Q. *schneckii* is of a light green color and quite deeply lobed, and the lobes are more divided into smaller lobes than any of the others. It is much thinner and hence more flexible than any of the others. The leaf of Q. *coccinea* is of a dark green color with a glossy upper surface. It is longer than the others and has fewer lobes, which are nearer at right angles to the midrib than in any of the others. Q. *macrocarpa* has a leaf quite different from any of the others. It is of a light green color and has many hairs on the lower side. The young leaves of all these oaks have hairs on both the upper and lower surfaces. The comparative sizes and shapes are shown in figures 1, 2, 3 and 4.

The Bleached Leaf.

One of the first differences noticed between the leaves of the different oaks was the time required to bleach the leaves. The leaf of Q. *macrocarpa* was cleared in the chloral hydrate in about two days, and took about the same time to clear in the potassium hydrate. The leaf of Q. *schneckii* and Q. *coccinea* was cleared in the chloral hydrate in about five days and in the potassium hydrate in two days. The leaf of Q. *rubra* was cleared in the chloral hydrate in seven days and in the potassium hydrate in three days, thus taking about twice as long to bleach as it took to bleach the leaf of Q. *macrocarpa*. The venation of the leaves was examined in the bleached leaves, but no constant difference could be noticed.

Upper Epidermis.

There is a noticeable difference in the size of the upper epidermal cells. There being 2500 cells per square millimeter in the leaf of Q. *macrocarpa*, 1900 in the leaf of Q. *schneckii* and Q. *coccinea*, and about 1650 cells per square millimeter in Q. *rubra;* the greatest difference being between Q. *macrocarpa* and the others, thus easily distinguishing Q. *macrocarpa* from the others. (See figs. 5, 6, 7 and 8.) A cross section of the leaf shows the upper epidermis of Q. *ruba* to be the thickest, being about .03 mm. thick. The upper epidermis is about .028

mm. thick in Q. *coccinea*, .02 mm. in Q. *schneckii*, and about .016 mm. in Q. *macrocarpa*. The cell walls of Q. *rubra* are also slightly thicker than in any of the others. (For the comparison, see figs. 13, 14, 15, 16.)

Leaf Parenchyma.

The palisade cells of Q. *macrocarpa* are slightly more numerous than in any of the others, there being 22,000 per sq. mm. in Q. *macrocarpa*, 21,100 per sq. mm. in Q. *rubra*, 20,200 per sq. mm. in Q. *schneckii*, and 16,600 per sq. mm. in Q. *coccinea*. (See figs. 5, 6, 7, 8.) The cross section of the leaf shows the palisade cells of Q. *schneckii* to be shorter than the others, being .035 mm. long, while in the other leaves they are about .045 mm. in length. This seems to account for the thinness of the leaf of Q. *schneckii*. (See figs. 13, 14, 15 and 16.)

Lower Epidermis.

The sizes of the cells in the lower epidermis are nearly the same in all except in Q. *macrocarpa*, there being 10,960 cells per sq. mm. in Q. *macrocarpa*, and in the others they run thus from 4920 in Q. *schneckii* to 4900 in Q. *rubra* and 4870 per sq. mm. in Q. *coccinea*. The thicknesses of the lower epidermis are various in the different species. In this respect any measurements give Q. *rubra* .016 mm., Q. *schneckii* .015 mm., Q. *coccinea* .012 mm., and Q. *macrocarpa* .010 mm. The lower epidermis of Q. *macrocarpa* also has persistent hairs in tufts, while in the others the hairs fall off when the leaves are quite young.

Stomata.

The stomata are found on the lower side of the leaf. They vary among the different species as to number, size and shape. The number of stomata per square millimeter was found by taking the average of twenty-five fields from various parts of the leaf, except where a vein occupied a whole field such a field was not counted. The fields counted were .09 sq. mm. in area. The number of stomata per square millimeter was found to be 375 in Q. *schneckii*, 500 in Q. *coccinea*, 515 in Q. *rubra*, and 819 in Q. *macrocarpa*. The stomata of Q. *schneckii* are of two sizes, the larger size being the more numerous and of elliptical shape, while the smaller size are almost round. The stomata of Q. *rubra* and Q. *coccinea* are almost round, but in Q. *macrocarpa* the stomata are quite elliptical and smaller than in any of the others. The length of the opening is nearly one-half the length of the guard cells in *Q. schneckii*, about two-fifths the length of the guard cells in *Q. rubra* and *Q. coccinea* and one-third the length of the guard cells in *Q. macrocarpa*. The length of the larger stomata of *Q. schneckii* is about .03 mm., and the length of the smaller sized stomata of *Q. schneckii* is about the same as the length of the stomata of *Q. rubra* and *Q. coccinea*, which is about .024 mm. In *Q. macrocarpa* the stomata are about .023 mm. long but are much narrower than in the others, hence they look much smaller. The proportionate lengths and shapes are shown in figures 9, 10, 11, 12.

Leaf Cross-section.

In the cross-section of the leaves it is noticed that the leaf of *Q. rubra* is slightly thicker than the others, it being .16 mm. thick, while *Q. coccinea* is about .15 mm. thick and *Q. schneckii* and *Q. macrocarpa* are about .13 mm. thick. The thinness of the leaf of *Q. schneckii* seems to be due to the thinness of the palisade parenchyma, and the thinness of the leaf of *Q. macrocarpa* seems to be due to the thinness of the spongy parenchyma. The spongy parenchyma, as well as the palisade parenchyma, is more compact in *Q. macrocarpa* than in the others. (See figs. 13 to 16, inclusive.)

Midrib.

In the cross-section of the midrib there are three layers of phloëm tissue, a fact characteristic of the oaks (Solereder). There is also xylem tissue with the middle layer of phloëm. The xylem is above the phloëm in the middle layer, or the phloëm is said to face the upper surface of the leaf. In tracing this middle layer of xylem and phloëm from the base of the leaf to the apex it is found that the xylem disappears, or unites with the upper layer of xylem before the phloëm does. The middle layer of phloëm always unites with the upper layer of phloëm, but in different ways in the different species, and also at different distances from the base of the leaf.

In *Q. rubra* this layer of phloëm usually extends about half the distance from the base to the apex. At this point the last or uppermost large vein usually branches off from the midrib. The upper layer seems to divide in the middle and the ends bend down. At the same time the middle layer of phloëm divides longitudinally, and the inner ends bend up to meet the ends of the upper layer which are bent down. This middle layer of phloëm also extends out into a few of the larger side veins, but only to a distance of about 4 mm. This method and place of ending was the same in the three trees of *Q. rubra* studied. (Figs. 17-19.)

In *Q. schneckii* this middle layer of phloëm extends about two-thirds the distance from the base to the apex of the leaf, which is usually the point where the vein of the upper largest lobe separates from the midrib. The middle of the phloëm ends by forming the partition between the midrib and the side vein branching from it. This usually unites with the upper layer just before the partition is formed between the vein and the midrib. The upper layer of phloëm bends down in the middle to unite with this middle layer of phloëm, which is almost divided longitudinally to unite with the two portions of the lower phloëm layer which form the lower layers of the midrib and vein. This middle layer of phloëm also extends out into the side veins about one-half the distance to the apex of the lobe. These characteristics were the same in the three trees of *Q. schneckii* studied. (See figs. 23-25.)

In *Q. coccinea* this middle layer does not end with the larger side veins as was the case with the other leaves studied. It extends about three-fourths the distance to the apex of the leaf. It first divides longitudinally, then each part unites with the outer ends of the upper layer. This middle layer extends up the side veins about two-thirds of their length. (See figs. 20, 21, 22.)

In *Q. macrocarpa* this layer of phloëm extends nearly three-fourths the distance to the apex of the leaf, where small veins branch off. It forms the partition between the veins and the midrib, and there are usually two veins branching off at the same place or nearly so. (See figs. 26 to 30.) This phloëm extends into only the two large side veins, and extends up them about two-thirds the distance to the apex of the lobes.

STEM.

In cross sections of the current year's growth of the stem the division into vascular bundles is more noticeable in *Q. ruba* and *Q. coccinea* than in *Q. schneckii* and *Q. macrocarpa*. In *Q. macrocarpa* the cork-cambium usually produces enough cork in the first year's growth to break up the epidermis, while in the others the epidermis remains unbroken for at least five years, and usually much longer. The number of water tubes per unit area is much less in *Q. macrocarpa* than in the others. There is the largest number of water tubes in *Q. schneckii.* It also has a smaller proportion of pith and parenchyma of the cortex than the others. The bast and phloëm in *Q. schneckii* and *macrocarpa* are almost entire, while in the others the bast and phloëm are divided. (See figs. 31 to 34.)

Cross sections of the older stems show that the water tubes in *Q. schneckii* occupy a much larger space and are more scattered throughout each year of growth than in any of the others. In *Q. rubra,* and especially *Q. coccinea,* the water tubes are usually found in the first part of each year's growth, and much less scattered through the whole year's growth than in *Q. schneckii.* In *Q. macrocarpa* there are not so many water tubes and they are not as large or disperse as in the others. (Figs. 43 to 46.) In tangential sections there is a difference in the number of medulary rays per unit area. Thus in *Q. macrocarpa,* which has the largest number per sq. cm., there are about 108 rays per sq. cm. The rays are also shorter and broader than in the others. In *Q. rubra* there are 96 rays per sq. cm. A large per cent of these are longer than in *Q. macrocarpa,* but the per cent of long rays is not as large as in *Q. coccinea,* which has the least number of rays per sq. cm., there being about 57 per sq. cm. The rays in *Q. coccinea* are also more slender. In *Q. schneckii* there are 75 rays per sq. cm., and no very long rays. (Figs. 47 to 50.)

Macerations of the wood and bast fibers were made by gently heating sections of each in Schultze's macerating fluid, in order to study the comparative lengths of the fibers. The wood fibers of *Q. macrocarpa* are on an average shorter than the others, all of which are about the same length; however, those of *Q. schneckii* are slightly broader than the others. The bast fibers are on an average about the same length, except in *Q. coccinea,* where they are about twice as long as in the others. Thus in *Q. coccinea* the bast fibers are as long as 1.4 mm.; in the others they average about .6 mm. in length. (Figs. 35 to 42.) There was no noticeable difference in size or character of the cells of the wood parenchyma of these four oaks.

ACORNS.

The size and structure of the acorns is noticeably different in all of these oaks; yet there is a somewhat close resemblance between the acorns of *Q. rubra* and *Q. schneckii.* The cup in *Q. schneckii* is deeper and has coarser bracts; the acorn is not so large, and is more pointed. The acorn of *Q. macrocarpa* is much larger than the others, and the acorn of *Q. coccinea* is much smaller. The shell of the acorn of *Q. rubra* is thicker than the others. The inner surface is covered with hair-like outgrowth in all except in *Q. macrocarpa,* which, however, has a thin layer of cells forming a lining membrane. The shell wall of *Q. coccinea* is much thinner than the others. (Figs. 55 to 58, inclusive.)

The starch grains were larger in *Q. rubra* and *Q. schneckii,* being about .0076 mm. in diameter, and in the others about .0062 mm. The acorns are more numerous on the branches of *Q. coccinea* and *Q. rubra.* For relative sizes and shapes see figs. 51 to 54.

CONCLUSIONS.

From this study of the oak it appears that there are enough differences in the various tissues of the oaks to enable one to distinguish the species by histological methods. There were found differences in the leaf sufficient to distinguish the different species, and in addition to this there are differences in the stem and acorns which would enable one to further distinguish the species.

Botanical Laboratory.
University of Kansas.

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CONTENTS:

ANATOMY OF *Platanus occidentalis*, ... *Maude Marie Baird.*

A thesis submitted to the Department of Botany and Faculty of the Graduate School in partial fulfillment of the requirements for the Master's Degree.

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Anatomy of *Platanus occidentalis.*

MAUDE MARIE BAIRD.

Plates LXV-LXXVI.

THE natural habitat of *Platanus occidentalis* is the flood plain of streams. The tree from which the material for this work was obtained is growing upon the campus of the University of Kansas, on prairie land which, though sheltered from the south winds, is at a considerable elevation above the Kansas river a half mile away, so that its water supply is less than it would receive in its natural habitat. This condition may have affected its anatomical structure to some extent. Miss Anna M. Starr ('97) has shown that there is a marked difference between members of a species of plant growing in mesophytic situations and members of the same species growing in xerophytic situations. She gives the following interesting comparison of *Platanus occidentalis* growing in a swamp habitat and the same plant which, by increased length of stem, has been able to keep pace with a moving dune which is passing over the place where it is growing.

In the following table S stands for the swamp form and X stands for the dune form:

LEAF.

	S.		X.	
Thickness	152 μ		199 μ	
Thickness of upper epidermis	23	= 15%	25	= 12%
Depth of palisade	52	= 34%	63	= 32%
Depth of sponge	62	= 41%	92	= 46%
Tickness of lower epidermis	16	= 10%	19	= 10%
Outer wall of epidermis	4		2.8	

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STEM.

	S.	*X.*
Number of vessels in the octant	66	69
Average diameter of the larger vessels	35 μ	46 μ
Thickness of walls of vessels	3.2	3.2
Thickness of walls of fibers	5	5
Lumen of the fibers	6.4	7
Number of growth rings	3	2
Sclerenchyma	100	116
Collenchyma	56	108

These tables show that, in comparison with the swamp form, the xerophytic form has thicker leaves, larger and more numerous water tubes in the stem, and more strengthening tissue in the bark.

ANATOMY.

The Leaf.

The leaves when young are very densely covered with two kinds of hairs. One sort (fig. 1) has a central axis about .24 mm. long, consisting of a straight row of elongated cells. This axis bears branches in whorls of three or four, each branch being composed of one or two cells, the longest of these cells being about .17 mm. in length. The other kind of hairs (fig. 2, *a*) are glandular, and consist of a short stalk of one cell surmounted by a globular cell about .026 mm. in diameter. The average length of the glandular hairs is .04 mm.

The lower epidermis is two-thirds as thick as the upper. The outer part of the wall of the upper epidermis is cutinized, as shown by its red color when stained with Sudan III. The inner part of the wall is cellulose. The entire outer wall of the lower epidermis is cellulose, but is covered by cuticle. The guard cells of the stomata are raised a little above the surrounding cells (fig. 5), and each guard cell has beneath it two or three relatively small epidermal cells as shown in cross section in fig. 5, *b*, and in surface view in fig. 6, *b*. These cells differ from the surrounding epidermal cells not only in size but also in cell contents, as will be shown in the chemical section of this paper. In all the leaves examined no stomata were found on the upper epidermis; but in Solereder (p. 780) the statement is made that stomata have been found upon the upper epidermis of this species. Upon the lower epidermis the stomata average 124 to the square millimeter. The average length of the openings of the stomata is .002089 mm. Using

Brown and Escombe's formula for finding the area of the stomatal openings per unit area of surface, $A = l \times w \times \frac{\pi}{4} \times$ number of stomata, we have $.006567 \times .00289 \times .7854 \times 124$ (number of stomata per sq. mm.) $= .01336$ sq. mm. = area of the stomatal openings per sq. mm.; that is, when the stomata stand open, the stomatal openings form about $1\frac{1}{3}$ per cent of the area of the lower leaf surface.

In the leaves which I studied there was only one layer of palisade cells, the usual layer just beneath the upper epidermis, and not an upper and lower layer as Solereder states (p. 780).

Fig. 7 is a surface view of a leaf embracing the epidermal, the palisade, and the vascular bundle systems. It shows well the free endings of the tracheids in the ground tissue of the leaf. The greatest distance between the ultimate endings of veins, or between an ultimate ending and the nearest vein, is not more than .08 mm. Some of the tracheids of the leaf have annular or scalariform thickenings (fig. 8, *t*), and others have transversely placed small oval bordered pits as in the tracheids of the wood. The border parenchyma of the veins of the leaf is composed of elongated cells with rather thick walls containing numerous pits (fig. 8, *k*). The midrib and the larger veins in the mature leaves have heavy strands of bast fibers, or these united into a ring, surrounding the group of vascular bundles (fig. 19, *b*.) Near the leaf blade the vascular bundles of the petiole are arranged in either two or three rings, one above the other, as shown in figure 20. Farther down the petiole the bundles are arranged as shown in figure 21. Just above the place where the petiole opens to include the bud the vascular bundles become divided into groups which form a single ring (fig. 23). The individual strands separate rather widely in the part of the petiole which incloses the bud (fig. 24), and enter the stem in this condition. Figures 25 to 28 show how these groups of bundles approach the ring of vascular bundles of the stem and occupy the leaf-trace gaps.

Figure 2 represents a cross section of a very young leaf. No cell walls have thickened excepting the walls of three or four tracheids. Figure 3 shows a cross section of the midrib of a somewhat older leaf. More differentiation of tissues is shown. The cells have increased, not only in number, but also in size. The walls of the cells which are to become bast fibers and the outer wall of the epidermal cells have not yet begun to thicken. Figure 4 shows part of a cross section of a mature leaf. The epidermis has become cutinized and the walls of the bast fibers have become thick and lignified.

The Peduncle.

The peduncle has remarkably thick, strong bundles of bast fibers just outside the phloëm (fig. 35, *g*, and fig. 36, *k*). These strands of bast persist long after the rest of the peduncle has disintegrated. The fruit hanging suspended by these flexible threads is tossed about by the wind. This seems to be a device for scattering the seeds when the balls of fruits begin to fall to pieces in the spring.

The Buds.

The buds (fig. 30) while forming are entirely inclosed in the bases of the petioles. In winter condition they are protected by cone-shaped scales (fig. 34). The outer scale is thick and hard. The next (fig. 34, *b*) is covered by a mass of resin through which project numerous hairs about 2 millimeters long. The inner epidermis of this scale contains in some places as many as 1200 crystals of calcium oxalate to the square millimeter. On removing this scale one leaf is exposed, but the remainder of the leaves and the inflorescence are inclosed in still another conical scale densely covered with straight hairs 2 millimeters long, consisting of a chain of about four elongated cells. If the bud contains an inflorescence, the inflorescence is terminal as shown in figure 31, and there is a series of foliage leaves below it. Such a limb continues its growth by a lateral bud in the axil of the last leaf. The peduncle which terminates the main axis is pushed to one side by the development of the axillary bud at its base, which then has the appearance and later performs the function of a terminal bud. Usually a large, vigorous bud and one or two smaller buds are inclosed together in the same outer scales. The weaker buds usually do not develop. Some of them begin to unfold, and then fall. The terminal bud may contain one or two flower buds, one or two leaf buds, or both leaf and flower buds. The terminal bud may produce either pistillate or staminate flowers, and the same thing is true of the lateral buds.

Figures 37 to 42 show how the rings of vascular bundles of three separate buds merge into the one ring of the stem which bears them. Just below the terminal bud there is an enlargement of the stem. In this part of the stem there is an extra amount of pith, containing a rich store of food for the unfolding leaves of the following season. This pith is lignified and plain pitted. A mass of stone cells is found at one side of the stem in this region, just beneath the peduncle or just beneath the fundament of a flower bud which never develops.

The Bark.

There are bast fibers in the young bark, occurring usually in large bundles just outside the phloëm (fig. 44, *b*), but scattered fibers also occur in the same zone. These bast fibers vary from .3 millimeter to 1 millimeter in length. No secondary bast is formed. After a time phellogen is formed within the secondary phloëm, and the outer bark including all the bast fibers is shed, so that the bark on the older limbs contains no bast fibers. The walls of the cells of the medullary rays in the bark become much thickened and pitted. In the rays groups of lignified cells alternate with groups of unlignified cells. The part of the ray formed early in the season becomes lignified, while the part formed late remains cellulose. In both young and old bark masses of lignified parenchyma cells are scattered through a ground mass of cellulose parenchyma cells. The older bark contains a very large proportion of stone cells (fig. 46, *s*).

The Wood.

The wood (fig. 52) is composed of tracheal tubes, wood parenchyma, fiber tracheids, and the parenchyma of the medullary rays. There are no wood fibers. The greater part of the wood is composed of fiber tracheids. The tracheids, the shape of which is shown in figure 55, have an average length of .64 mm. and an average width of .0178 mm. These fibers have oval bordered pits placed obliquely (fig. 52, *k*). The wood parenchyma, of which there is little, has plain pits and lies next to the tracheal tubes. The largest tracheal tubes have a diameter as great as .075 mm. These tubes have oval bordered pits arranged in transverse rows (fig. 52, *w*). In a longitudinal section of one of the largest of these tubes there are as many as seven of these pits to the row. By performing the following experiment the length of the tracheal tubes was found to be 22 cm.:

The bark was removed from a straight shoot and the wood was covered with wax. One end was fitted with a glass funnel, and the other end was passed through a rubber cork into a side delivery flask almost full of water, the end of the shoot extending below the surface of the water. The air was then exhausted from the flask by means of a filter pump, and the shoot was shortened until bubbles of air streamed from the lower end. Mercury was then poured into the funnel and the shoot was shortened a little at a time until a spray of tiny drops of mercury began to fall from the lower end of the shoot, showing that some of the tracheal tubes extended the entire length of the shoot. The shoot used was four years old at one end and five years old at the other.

In tangential section there are about three medullary rays to the square millimeter. These rays vary in width from one to twelve cells, or from .034 to .216 mm., and in vertical length from nine to one hundred forty cells, or from .15 mm. to 2.42 mm. The cells of the rays are plain pitted and have an average width vertically and tangentially of .03 mm., and vary in length from .013 mm. to .1 mm.

A longitudinal section was made through the region of the stem where the old wood joined the new wood (fig. 58). The xylem of the old wood was so perfectly connected with that of the new wood that no distinct line of division could be made out. However, there were shorter tracheids at the close of a year's growth, and there was a difference between the old and the new pith, the new pith being made up of larger cells and containing less stored food (fig. 59).

CHEMICAL TESTS ON CELL CONTENTS.

The Leaf.

Leaves that had been in bright sunlight for several hours brought in and tested at once for starch with iodine showed the presence of much starch.

Sections of the same leaves boiled with Fehling's solution showed great numbers of crystals of cuprous oxide, especially in the palisade cells.

Sections of leaves in alcannin over night showed no oil. Sections of leaves in Sudan III over night showed no oil, except here and there in the epidermis.

Sections of leaves were treated with methylene blue as a mucilage test. The palisade cells, certain cells in both the upper and lower epidermis, and from four to six cells beneath each stoma took the stain (figs. 5 and 6).

Sections of leaves which had been preserved in formaldehyde, when boiled in Fehling's solution, showed no cuprous oxide crystals, probably because the sugar had been soaked out of them. The same sections left in Fehling's solution over night showed a great number of crystals of cuprous oxide in masses. Left another night, the masses grew larger. This reaction indicates the probability of glucosides.

A section of the leaf boiled in Millon's reagent showed red color in the palisade cells and in some of the epidermal cells, indicating the presence of proteid. A section of leaf treated with concentrated nitric acid and dilute ammonia showed orange color for the contents of the palisade cells, some of the epidermal cells and some of the spongy parenchyma. This color reaction indicates proteid. Sections treated with ferric chloride turned very dark in color, especially the palisade cells, indicating that there is much tannin in the leaf.

The sticky substance between the two outer bud scales is not soluble in warm water; therefore it is not mucilage. It is soluble in alcohol and xylene, which shows that it is resin.

The Bark.

Sections treated with iodine showed the presence of much starch and proteid in the parenchyma of the bark.

Fehling's solution showed the bark to be loaded with sugar.

Alcannin and Sudan III showed granular particles having the appearance of oil.

Traces of volatile oil were obtained by distillation. Cold cover glasses were passed back and forth past the mouth of a tube in which small pieces of bark were being heated. The volatile oils passed over with the steam and were condensed on the cover glasses.

Methylene blue showed the presence of no mucilage.

Some sections were soaked two days in water. Part of these, boiled at once in Fehling's solution did not show crystals of cuprous oxide. The rest of the sections put into Fehling's solution and left in a warm oven over night were full of crystals in the region of the bark, indicating that there is probably much glucoside here.

Pieces of the bark were boiled in water. Some of the water boiled with Fehling's solution showed minute crystals of cuprous oxide. These were removed by filtering, more Fehling's solution was added, and the solution was left in an electric oven over night. No more crystals formed, showing that no glucoside soluble in hot water had been formed. A section boiled in Millon's reagent showed the cell contents red in some of the cells of the medullary rays, some in the phloëm, and many in the parenchyma of the bark. The cambium was a continuous line of red. Millon's reagent caused a heavy white precipitate in the parenchyma of the bark. Sections of the bark soaked in dilute potassium hydroxide, chloroform, alcohol, hot water, and salt water still gave the precipitate on being treated with Millon's reagent. A section soaked in dilute HCl and then treated with Millon's reagent gave no precipitate showing that salts either of calcium oxalate or calcium carbonate are present in the bark. A section was treated with HCl, and since no bubbles of gas were given off the precipitate must be due to calcium oxalate and not to calcium carbonate.

A section treated with nitric acid and then with ammonia showed the presence of much proteid in the bark beginning with the cambium. Ferric chloride, copper acetate, and chromic acid all showed the presence of tannin in some cells of the phloëm and in most of the parenchyma of the bark. Bits of bark were put into dishes containing alcohol, alcohol ether, and xylene. The next day bits of Swedish filter paper were put into the dishes and the fluid allowed to evaporate. Then part of the pieces of filter paper from each dish were put into alcannin and part into Sudan III, testing for oil, and part into copper acetate as a test for resin, with the following results:

I. Alcohol extract.
    a. In alcannin. Small red bodies in great numbers clinging to the fibers of the paper. Also many larger bodies composed of aggregations of the smaller ones.
    b. In Sudan III. Same sort of bodies stained bright red.
    c. In copper acetate. Same bodies stained a deep brown. Fibers of the filter paper also covered with a brownish-green film.

II. Alcohol-ether extract.
  a. In alcannin. Very numerous large bodies with irregular outline stained red.
  b. In Sudan III. Same bodies bright red.
  c. In copper acetate. Same bodies brown.

III. Xylene extract.
  a. Alcannin. Red patches in great numbers without definite outline.
  b. Sudan III. Same spots or patches stained a yellowish-red.
  c. Copper acetate. Fibers of the paper were covered with a grayish-green film. Denser patches between the fibers were of the same color.

Since the material extracted had every appearance of being oil when treated with alcannin and Sudan III, and since it did not become emerald green when treated with copper acetate, as resin would do, the conclusion would be that it was oil, but on being treated with Tunmann's saponifying reagent there was no saponification.

Wood.

The medullary rays and pith of young wood and the rays of older wood are loaded with starch of different kinds, as shown by the different shades of blue when stained with iodine. There is also starch in the wood parenchyma.

Fehling's solution showed that sugar is associated with starch in the pith, wood parenchyma and medullary rays.

Alcannin and Sudan III showed no oil in the wood.

Bits of wood were put into alcohol, alcohol-ether, and xylene, and the same tests were made as the last recorded above for the bark. Nothing was extracted by these solvents, showing that the wood does not contain oil or resin.

Methylene blue did not show mucilage. Glucoside tests showed the presence of no glucosides in the wood.

Millon's reagent did not give the reaction for proteid in a section of wood five years old. In young wood it showed occasional cells in the pith and medullary rays containing proteid.

A section of wood treated with nitric acid and ammonia on a slide showed occasional cells in pith and rays which contain proteid, such cells being stained a deep yellow.

Ferric chloride showed tannin in the wood parenchyma, and in some of the cells of the medullary rays and pith.

Botanical Laboratory,
University of Kansas.

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PLATE I.

FIG. 1.—Upper end of pneumatocyst, showing "bald-head" with the bases of the frond clusters.

FIG. 2.—View of kelp bed with bald-heads in foreground.

FIG. 3.—Upper end of pneumatocyst covered by *Lacuna* eggs.

FIG. 4.—Upper end of pneumatocyst, showing tracings made by *Lacuna* when eating.

FIG. 5.—Fragments of frond with several snails; badly eaten.

FIG. 6.—Float, showing appearance at close of experiment.

FIG. 7.—Colonies of *Membraniphora membranacea* on a frond.

PLATE I.

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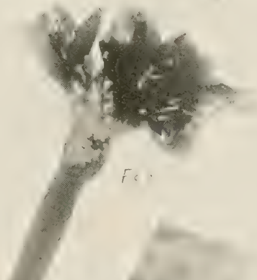
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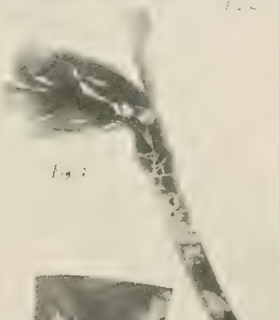
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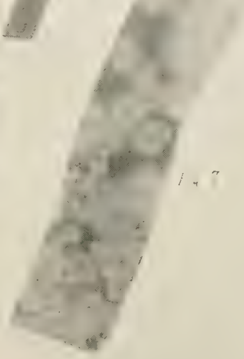
Fig 7

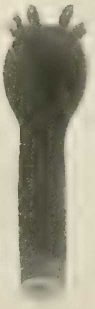
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Fig 5

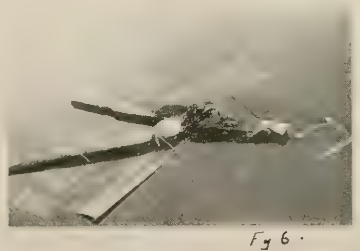
Fig 6.

PLATE II.

FIG. 1. Bleached leaf, showing venation. × 20. *a*, Water-storage tissue between the three largest veins.

FIG. 2. Diagram of a cross section of leaf, showing arrangement of tissue. × 70. *b*, Photosynthetic tissue; *c*, conducting tissue; *e*, water-storage tissue; *d*, midrib.

FIG. 3. Cross section of leaf. × 130. *f*, Epidermis; *g*, stoma; *h*, photosynthetic cells; *i*, vascular bundle; *j*, cells near the veins which contain chloroplasts; *k*, resin gland; *l*, resin duct; *m*, water-storage cells; *n*, tracheids.

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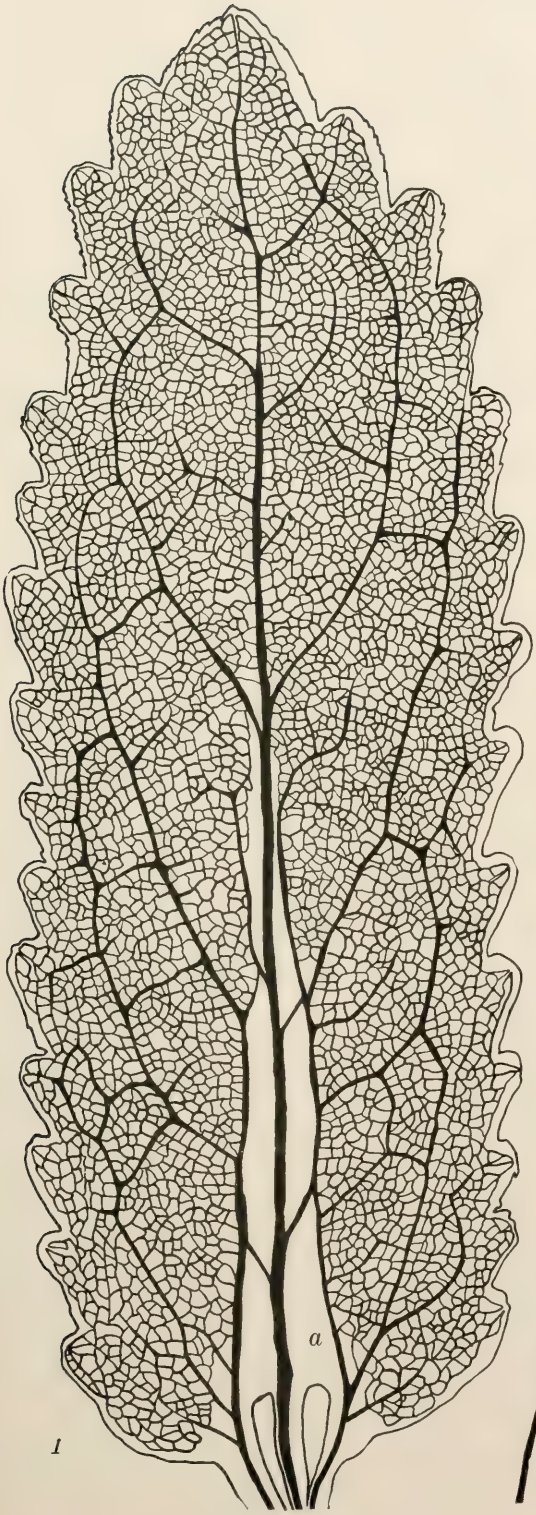
PLATE II.



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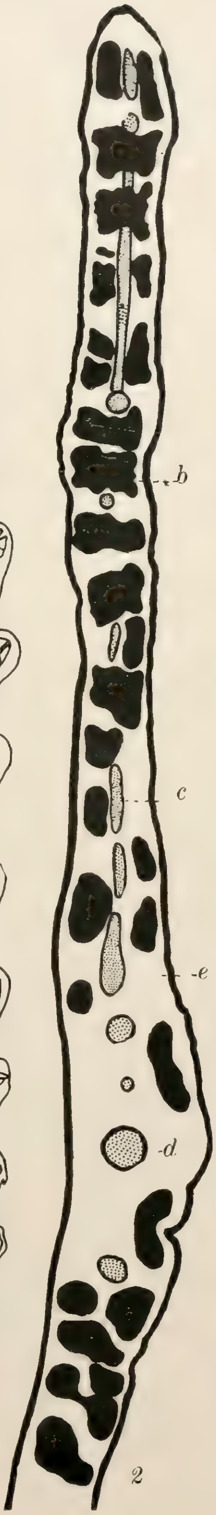

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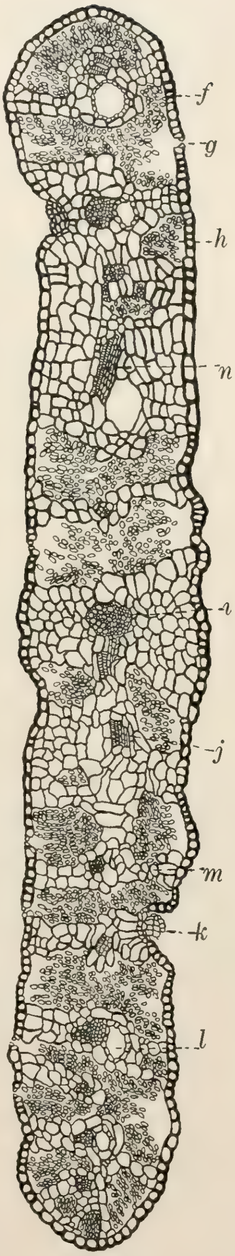

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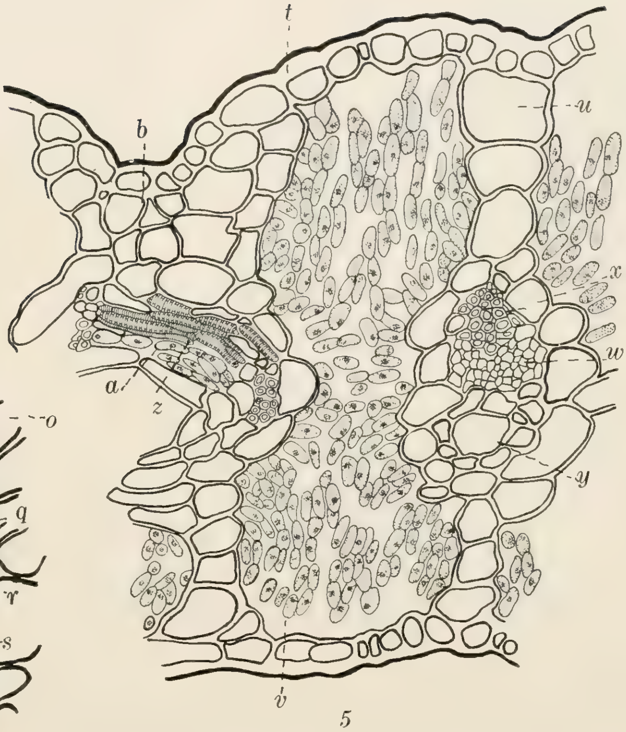
PLATE III.

FIG. 4. Midrib of leaf. × 500. *o*, Water-storage cells; *p*, phloem of midrib; *q*, conjunctive parenchyma separating xylem and phloem; *r*, tracheids; *s*, xylem parenchyma.

FIG. 5. Cross section of portion of leaf. × 500. *t*, Epidermis; *u*, water-storage cells; *v*, photosynthetic cells; *w*, phloem of a vascular bundle; *x*, xylem of a vascular bundle; *y*, resin duct, *z*, tracheids; *a*, photosynthetic cells; *b*, water-storage cells.

FIG. 6. Tangential section of leaf. × 300. *c*, Water-storage cells; *d*, photosynthetic tissue.

PLATE III.



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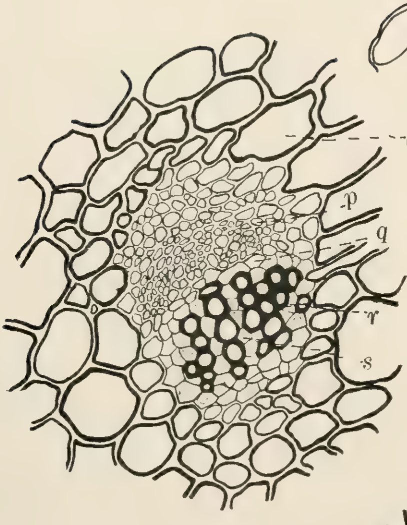

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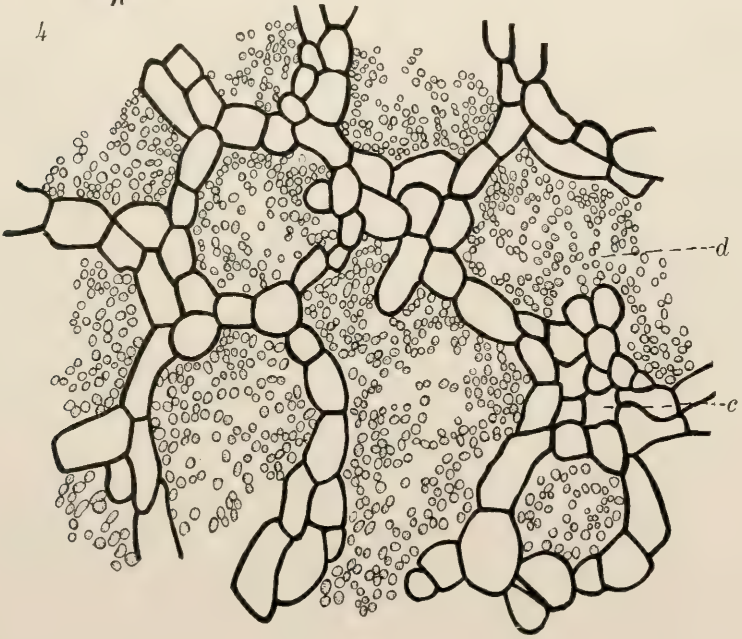

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PLATE IV.

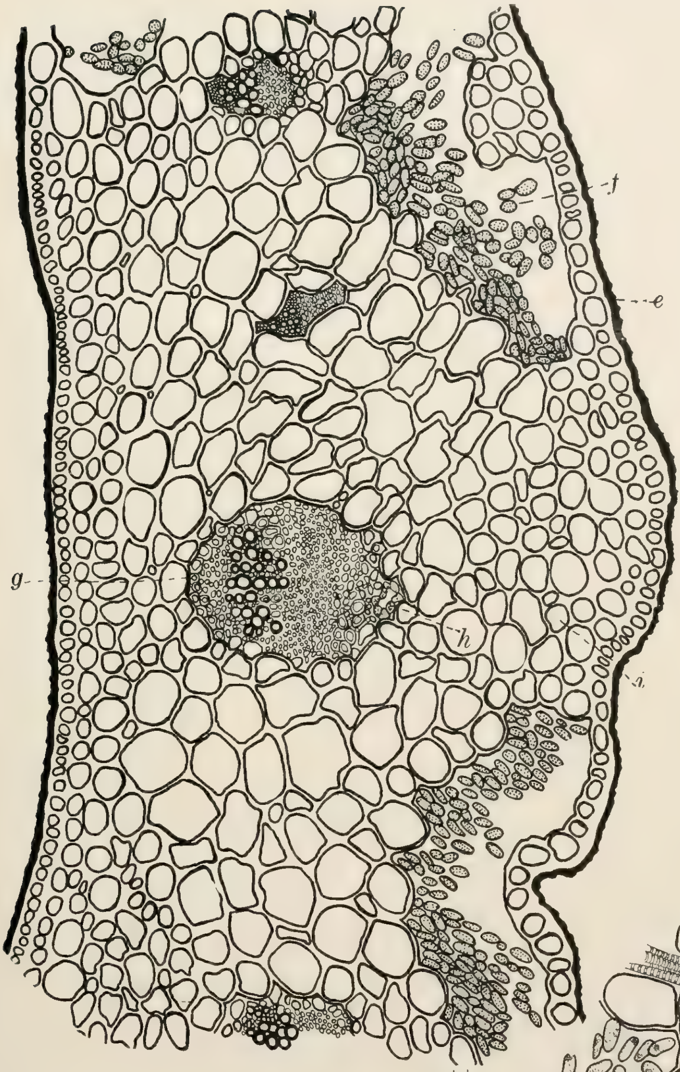
FIG. 7. Cross section of leaf through the midrib. × 300. *e*, Epidermis, showing cuticle; *f*, photosynthetic cells; *g*, midrib; *h*, resin duct; *i*, water-storage cells.

FIG. 8. Longitudinal section of resin duct. × 500. *j*, Starch sheath; *k*, cells of resin duct; *l*, collenchyma.

FIG. 9. Cross section of resin duct. × 500. *m*, Resin duct; *n*, bast; *o*, starch sheath; *p*, collenchyma.

FIG. 10. Tangential section of leaf. × 500. *q*, Photosynthetic tissue; *r*, veins; *s*, water-storage cells.

PLATE IV.



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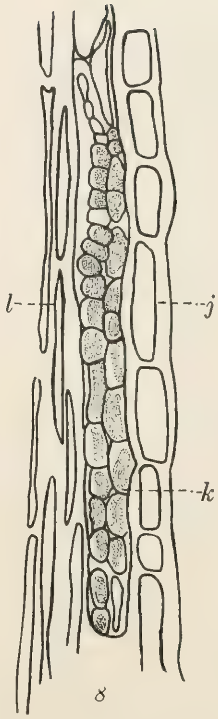

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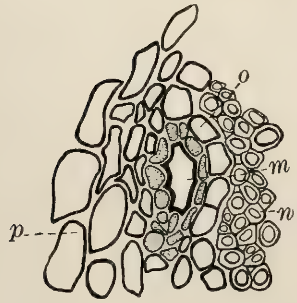

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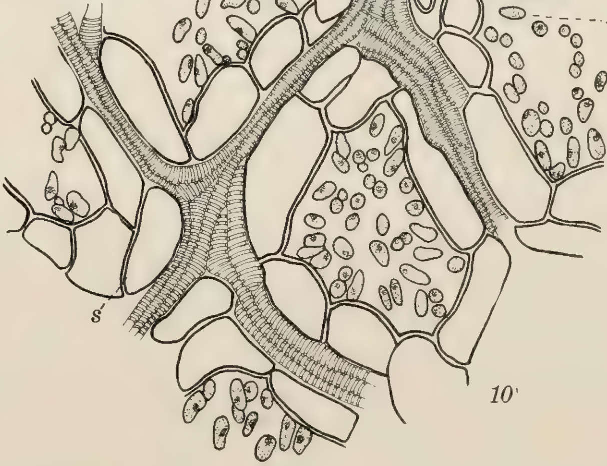

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PLATE V.

FIG. 11. Cross section of portion of a leaf, showing resin gland. × 500. *t*, Epidermis, showing cuticle; *u*, resin gland; v, photosynthetic cells; *w*, water-storage cells; *x*, xylem of a vascular bundle.

FIG. 12. Tangential section of leaf, showing cross section of resin gland. × 500. *y*, Water-storage cells; *z*, photosynthetic cells; *a*, resin gland.

FIG. 13. Tangential section through the tip of the tooth on the margin of the leaf. × 500. *b*, Resin glands; *c*, tracheids; *d*, water-storage cells; *e*, tracheids; *f*, water-storage cells.

FIG. 14. Cross section through the tip of a tooth on the margin of the leaf. × 300. *g*, Resin glands; *h*, water-storage cells; *i*, tracheids; *j*, photosynthetic cells.

FIG. 15. Composite drawing of bleached leaf. × 500. *k*, Epidermal cells; *l*, veins; *m*, stoma; *n*, water-storage cells.

FIG. 16. Epidermis of leaf. × 500. *o*, Stoma; *p*, epidermis; *q*, photosynthetic cells.

FIG. 17. Photosynthetic cells. × 500. *t*, Nucleus; *s*, chloroplasts.

FIG. 18. Epidermis of leaf. × 500. *u*, Epidermal cell; *v*, stoma; *w*, guard cell.

FIG. 19. Epidermis of leaf. × 500. *x*, Epidermis; *y*, guard cell; *a*, cuticle on inner wall of guard cell.

FIG. 20. Tracheids from leaf. × 500. *b*, Spiral tracheids; *c*, pitted tracheids; *d*, tracheids with elongated pits.

PLATE V.

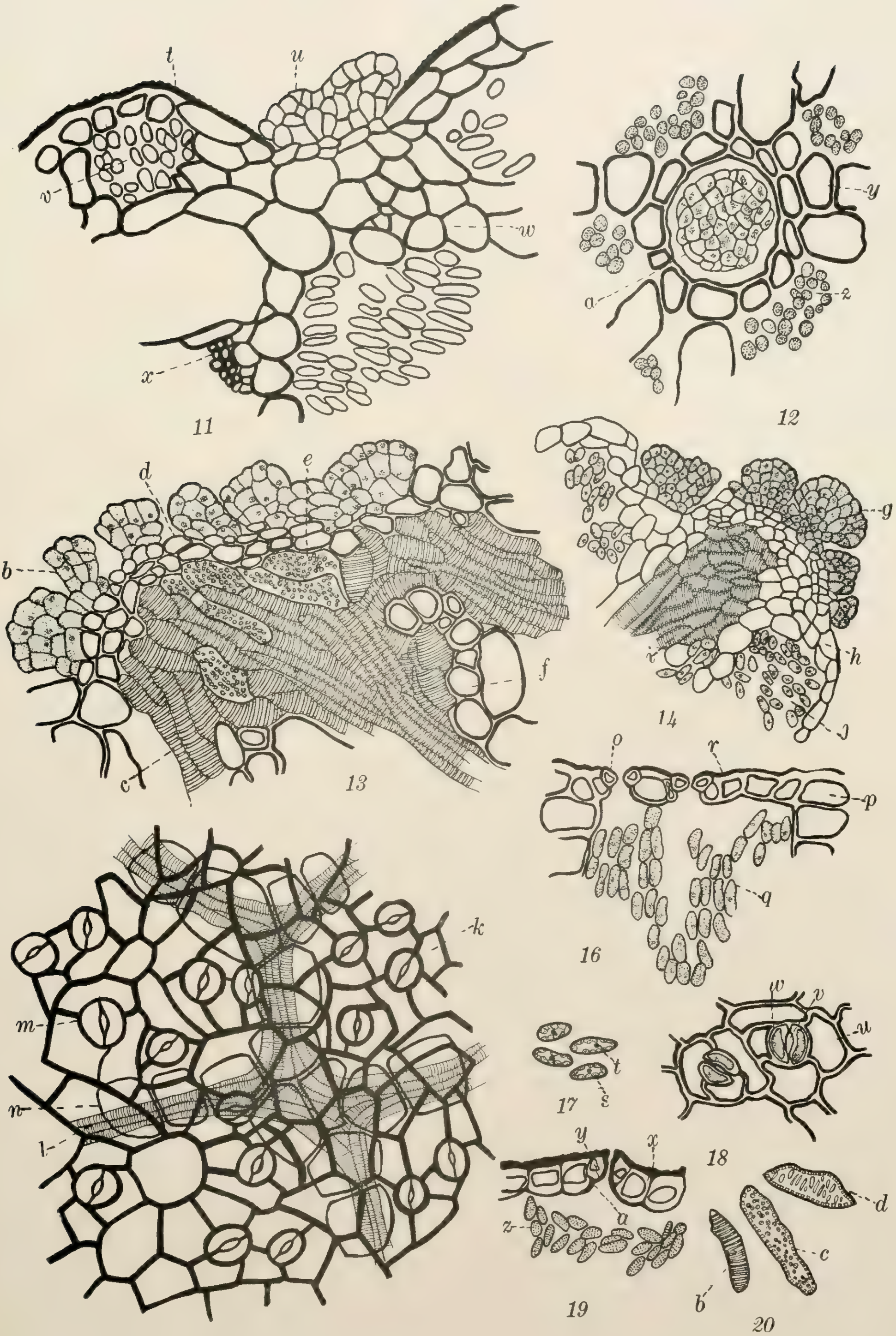
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PLATE VI.

FIG. 21. Protuberance on stem. × 500. *e*, Epidermis; *f*, collenchyma.

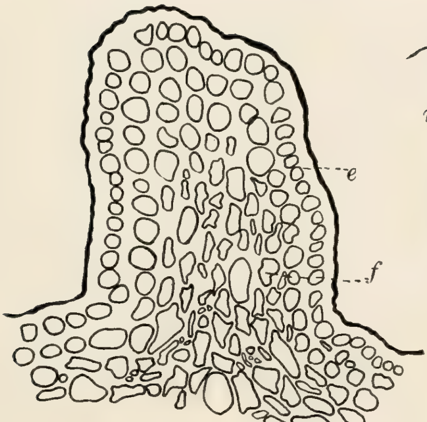
FIG. 22. Section of stem from epidermis to pith. × 500. *g*, Pith, *h*, tracheal vessel; *i*, phloem; *j*, wood fibers; *k*, starch sheath; *l*, epidermis; *m*, collenchyma; *n*, bast; *o*, parenchyma between bast groups.

FIG. 23. Longitudinal section of pith. × 500.

FIG. 24. Cross section of pith. × 500.

FIG. 25. Diagram of stem. × 70 *p*, Epidermis; *q* collenchyma; *r*, phloem; *s*, bast; *t*, xylem; *u*, pith.

PLATE VI.



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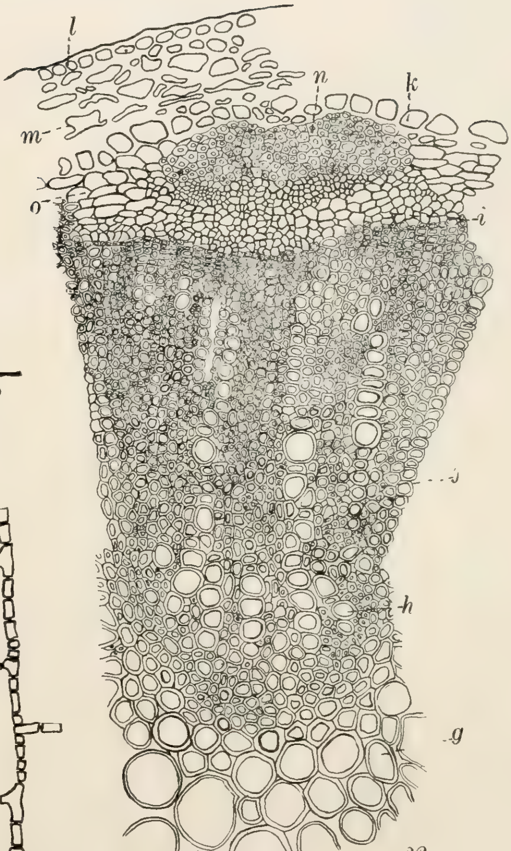

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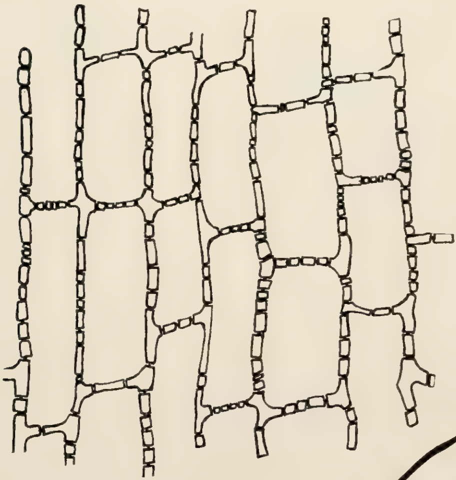

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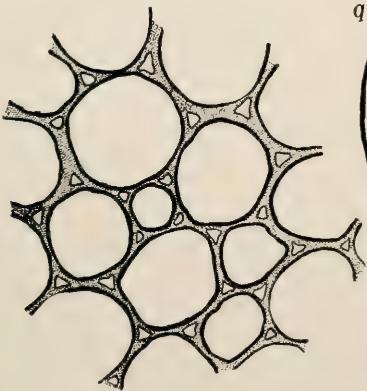

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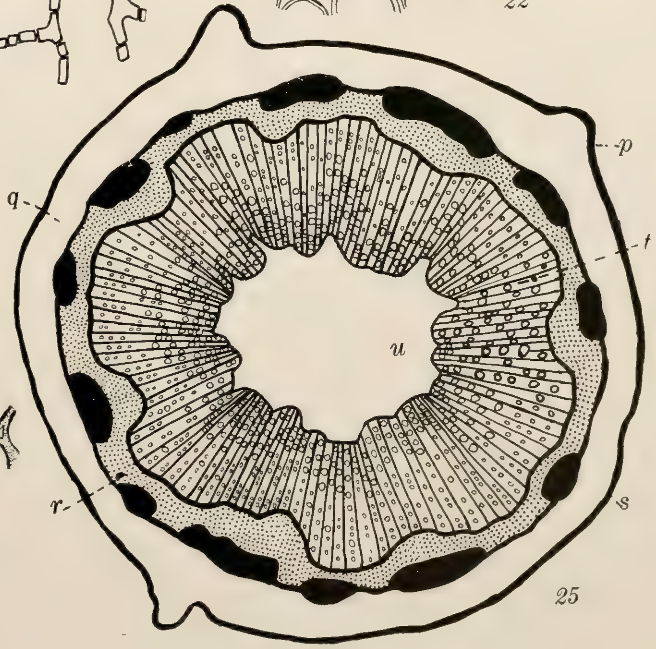

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PLATE VII.

FIG. 26. Base of vascular bundle. × 500. *v*, Wood fibers; *w*, tracheal tubes; *x*, lignified pith cells; *y*, xylem parenchyma.

FIG. 27. Medullary ray. × 500. *z*, Xylem; *a*, pith; *b*, medullary ray cells; *c*, cells resembling wood fibers.

FIG. 28. Cross section of portion of cortex from young stem. × 500. *d*, Cuticle; *e*, epidermis; *f*, collenchyma.

FIG. 29. Tracheal vessels. × 500. *g*, Pitted vessel; *h*, vessel with spiral thickenings.

FIG. 30. Cross section of phloem. × 500.

FIG. 31. Cross section of bast. × 500.

FIG. 32. Longitudinal section of wood fibers. × 500.

FIG. 33. Longitudinal section of wood parenchyma. × 500.

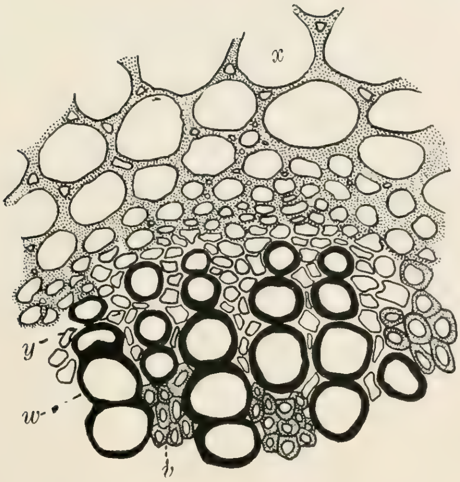
FIG. 34. Cross section of portion of cortex of old stem. × 500. *i*, Epidermis; *j*, collenchyma.

FIG. 35. Diagram of flower scale. × 120. *k*, Water-storage tissue; *l*, vein; *m*, photosynthetic tissue.

FIG. 36. Cross section of tissue separating two bast groups. × 580. *n*, Collenchyma; *o*, bast; *p*, starch sheath, showing portion not lignified; *q*, thin-walled cells separating bast groups.

FIG. 37. Longitudinal section through cortex. × 300. *r*, Epidermis; *t*, collenchyma; *s*, starch sheath.

PLATE VII.



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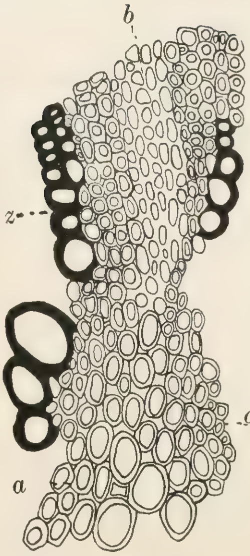

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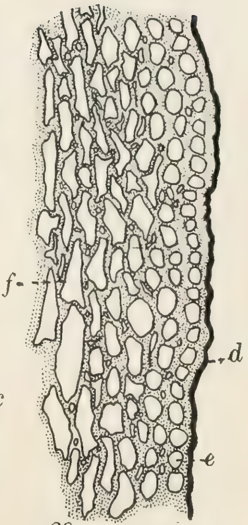

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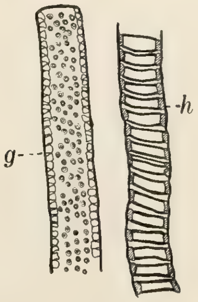

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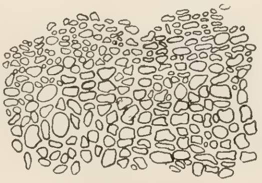

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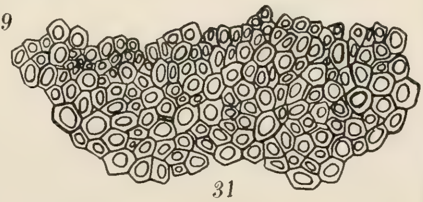

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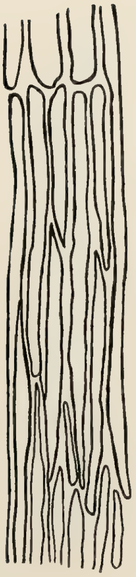

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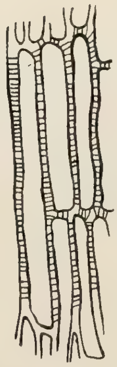

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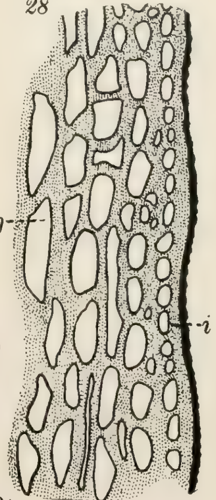

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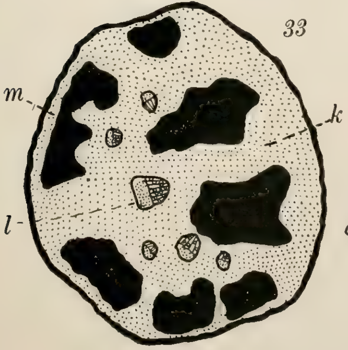

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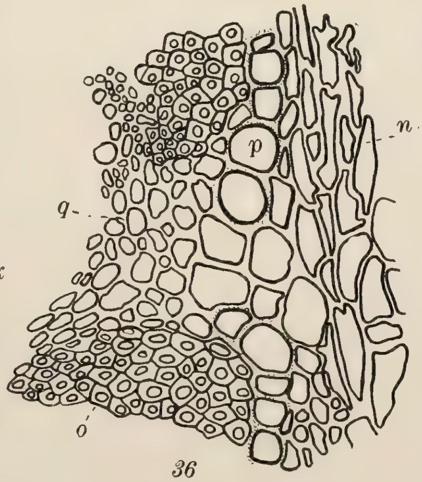

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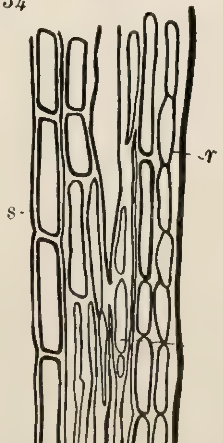

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PLATE VIII.

FIG. 1. Typical leaf of No. 1, average size. × ½.

FIGS. 2 and 3. Prevailing types of leaves of No. 2. × ½.

FIGS. 4 and 5. Prevailing types of leaves of No. 3. × ½.

FIGS. 5 and 6. Prevailing types of leaves of No. 4. × ½.

FIG. 8. Acorn of No. 1. A, side view; B, front view; C, seed; D, longitudinal section through cup. × 1.

FIG. 9. Acorn of No. 2. A, side view; B, front view; C, seed; D, longitudinal section through cup. × 1.

FIG. 10. Acorn of No. 3. A, side view; B, end view; C, seed; D, longitudinal section through cup. × 1.

FIG. 11. Acorn of No. 4. A, side view; B, front view; C, seed; D, longitudinal section through cup. × 1.

FIG. 12. Section through shell of acorn of No. 1. × 22½.

FIG. 13. Section through shell of acorn of No. 2. × 22½.

FIG. 14. Section through shell of acorn of No. 3. × 22½.

FIG. 15. Section through shell of acorn of No. 4. × 22½.

PLATE VIII.

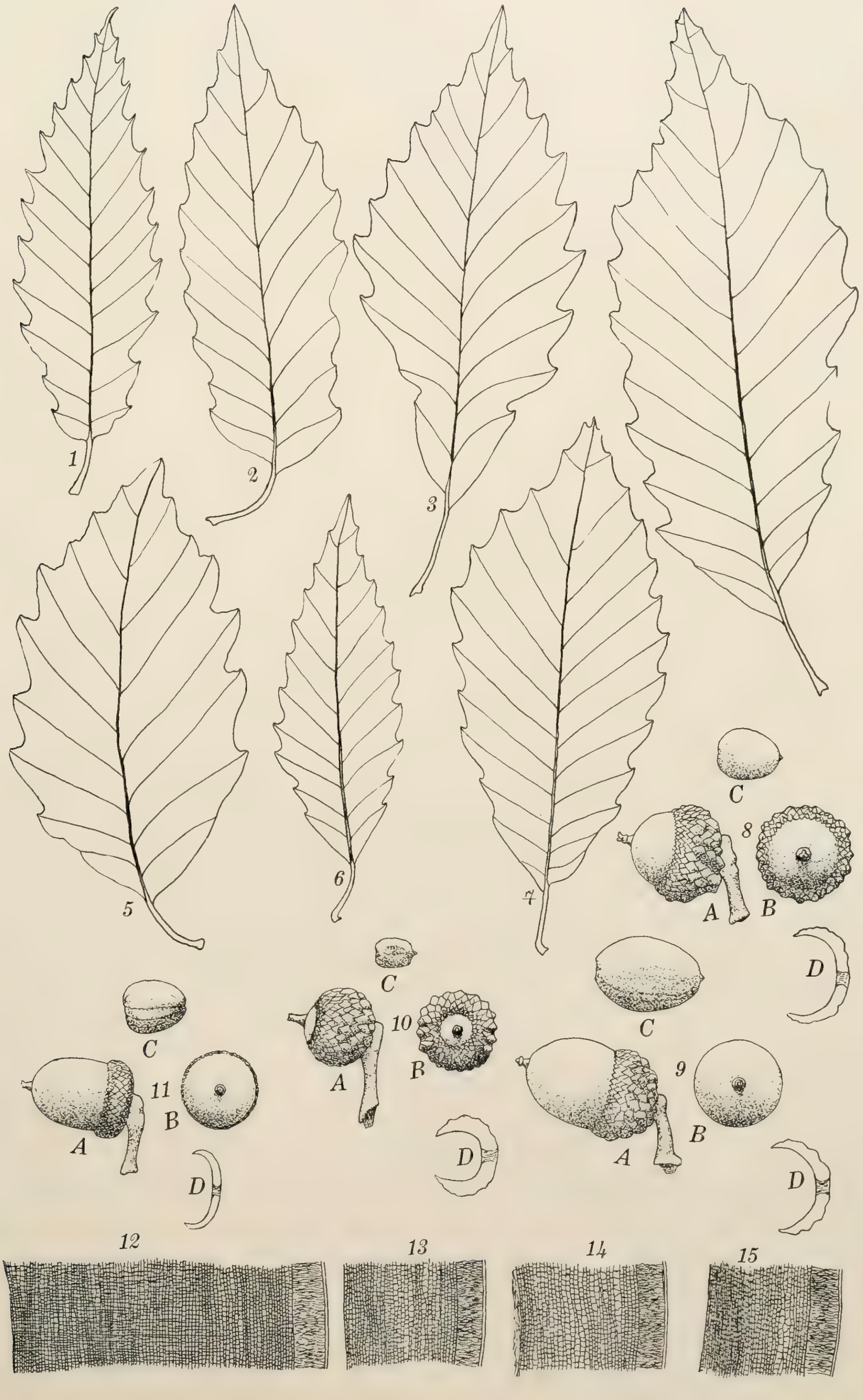
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PLATE IX.

FIG. 16. Stoma of *Q. muhlenbergii* (semidiagrammatical). Dotted line shows position of inner cell wall when cell is turgid. (p′, r′.)

FIG. 17. Longitudinal section of guard cell along line a-b, fig. 16.

FIG. 18. Cross section of stoma along line m-n, fig. 16. r-r′ shows movement in opening.

FIG. 19. Cross section of stoma along line x-y, fig. 16. p-p′ shows movement due to turgidity.

FIG. 20. Lower epidermis of leaf of *Q. rubra* about 2 days old, showing type and frequency of stomata. (Heavy lines.) × 405.

FIG. 21. Lower epidermis of leaf of *Q. rubra* about 7 days old, showing development of stomata. Same area as fig. 20. × 405.

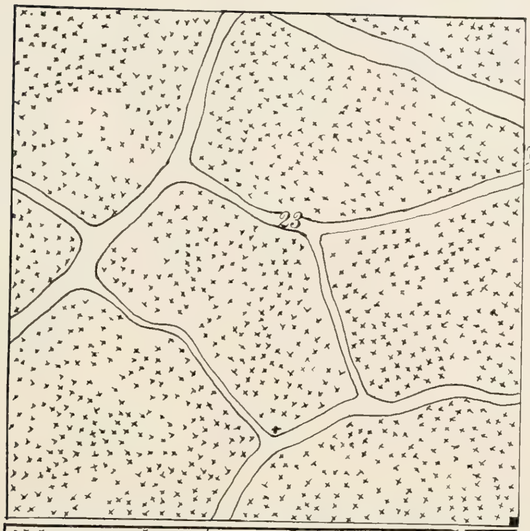
FIG. 22. Lower epidermis of leaf of *Q. rubra* at maturity, showing type and frequency of stomata. Same area as figs. 20 and 21. × 405.

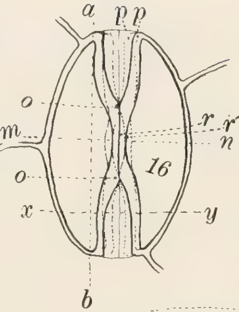
FIG. 23. Frequency of stomata on leaves of No. 1. 1 sq. mm. × 67. Black square in lower corner is 1 sq. mm.

FIG. 24. Frequency of stomata on leaves of No. 2. 1 sq. mm. of leaf surface. × 67.

FIG. 25. Frequency of stomata on 1 sq. mm. of leaf surface of No. 3. × 67.

PLATE IX.

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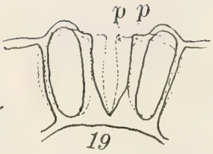

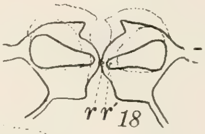

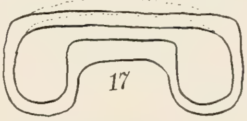

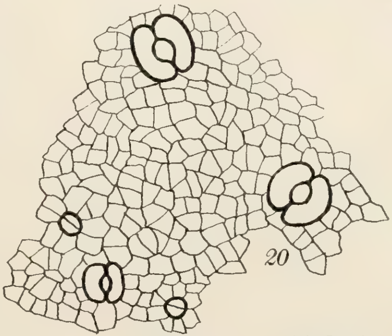

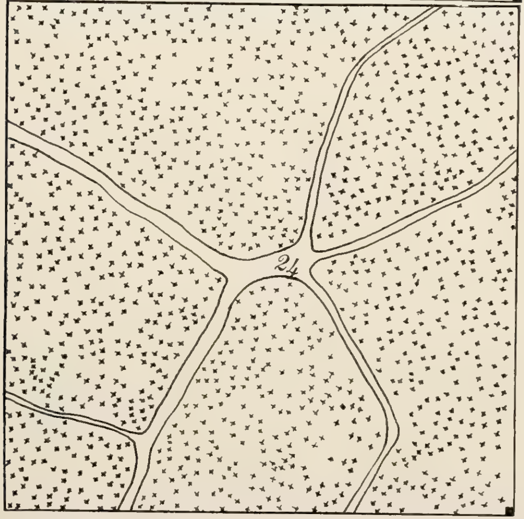

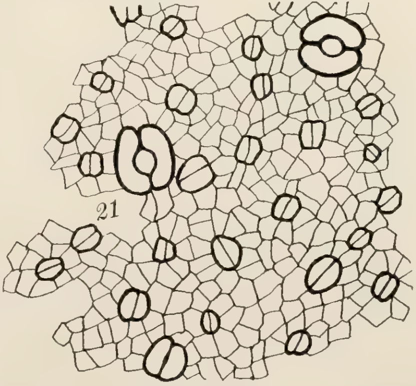

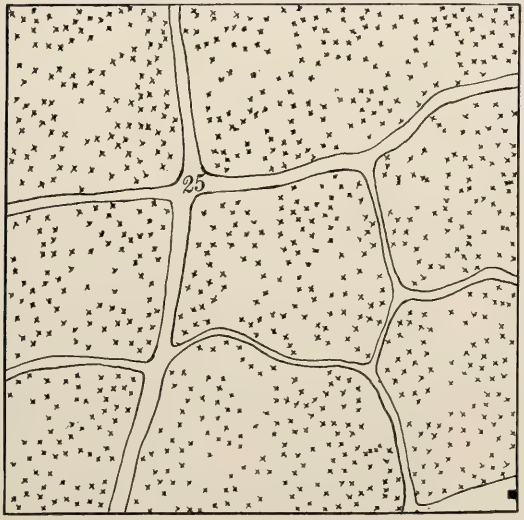

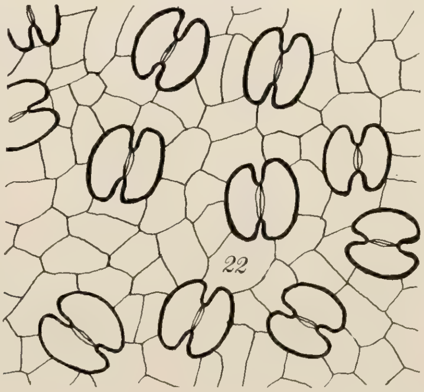

PLATE X.

FIG. 26. Frequency of stomata on 1 sq. mm. of leaf surface of No. 4. × 67.

FIG. 27. Cross section of midrib, No. 1 ⅔ length of leaf from base of blade. Bast stippled; phloem, black; xylem showing water tubes. × 23.

FIG. 28. Cross section of midrib, No. 2, same position as in No. 1 (fig. 27), showing persistence of middle bundle. × 23.

FIG. 29. Cross section of midrib of No. 3, same position as in No. 1 (fig. 27).

FIG. 30. Cross section of midrib of No. 4, same position as in No. 1 (fig. 27).

FIG. 31. Showing manner of ending of middle vascular bundle of midrib in Nos. 1, 2, and 3; e, position of bundles immediately above junction with vein; f, toii, stages in joining of middle bundle with upper bundle. Bast, stippled; phloem, black; xylem, showing water tubes (diagramatical).

FIG. 32. Modification of ending shown in fig. 31, also found in Nos. 1, 2, and 3.

FIG. 33. Showing manner of ending of middle bundle of midrib in No. 4. Middle bundle joining upper bundle at side.

FIG. 34. Cross section of petiole at base of blade, No. 1. Bast, stippled; phloem, black; xylem, showing water tubes; k, middle vascular bundle.

FIG. 35. Cross section of petiole of No. 2 at base of blade. × 23.

FIG. 36. Cross section of petiole of No. 3 at base of blade. × 23.

FIG. 37. Cross section of petiole of No. 4 at base of blade. × 23.

FIG. 38. Cells from seed of No. 1, showing starch grains. × 255.

FIG. 39. Cells from seed of No. 2, showing starch grains. × 255.

FIG. 40. Cells from seed of No. 3, showing starch grains. × 255.

FIG. 41. Cells from seed of No. 4, showing starch grains. × 255.

FIG. 42. Stone cells, acorn cup.

FIG. 43. Tracheids, acorn cup.

FIG. 44. Stone cells from shell of acorn.

PLATE X.

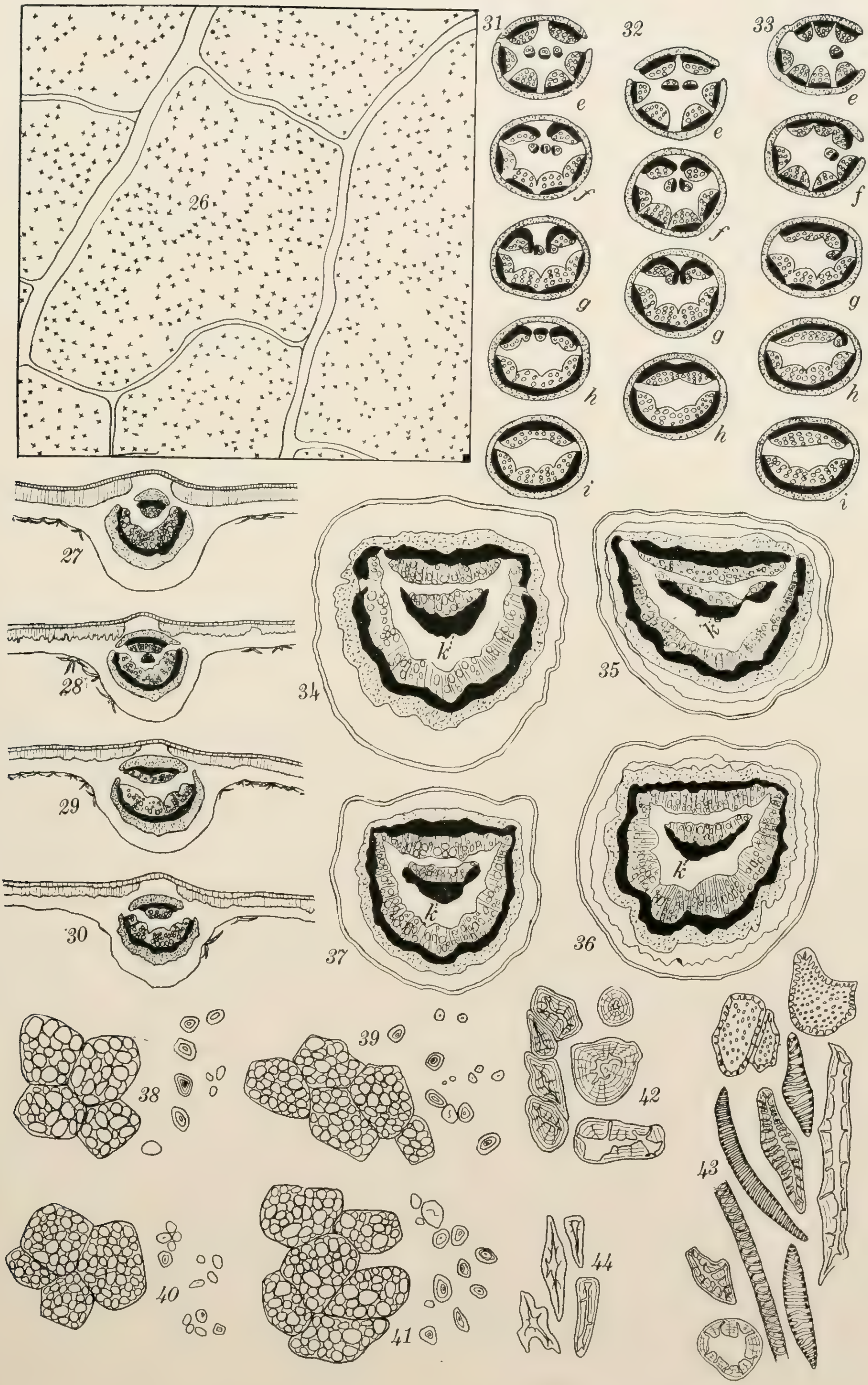
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PLATE XI.

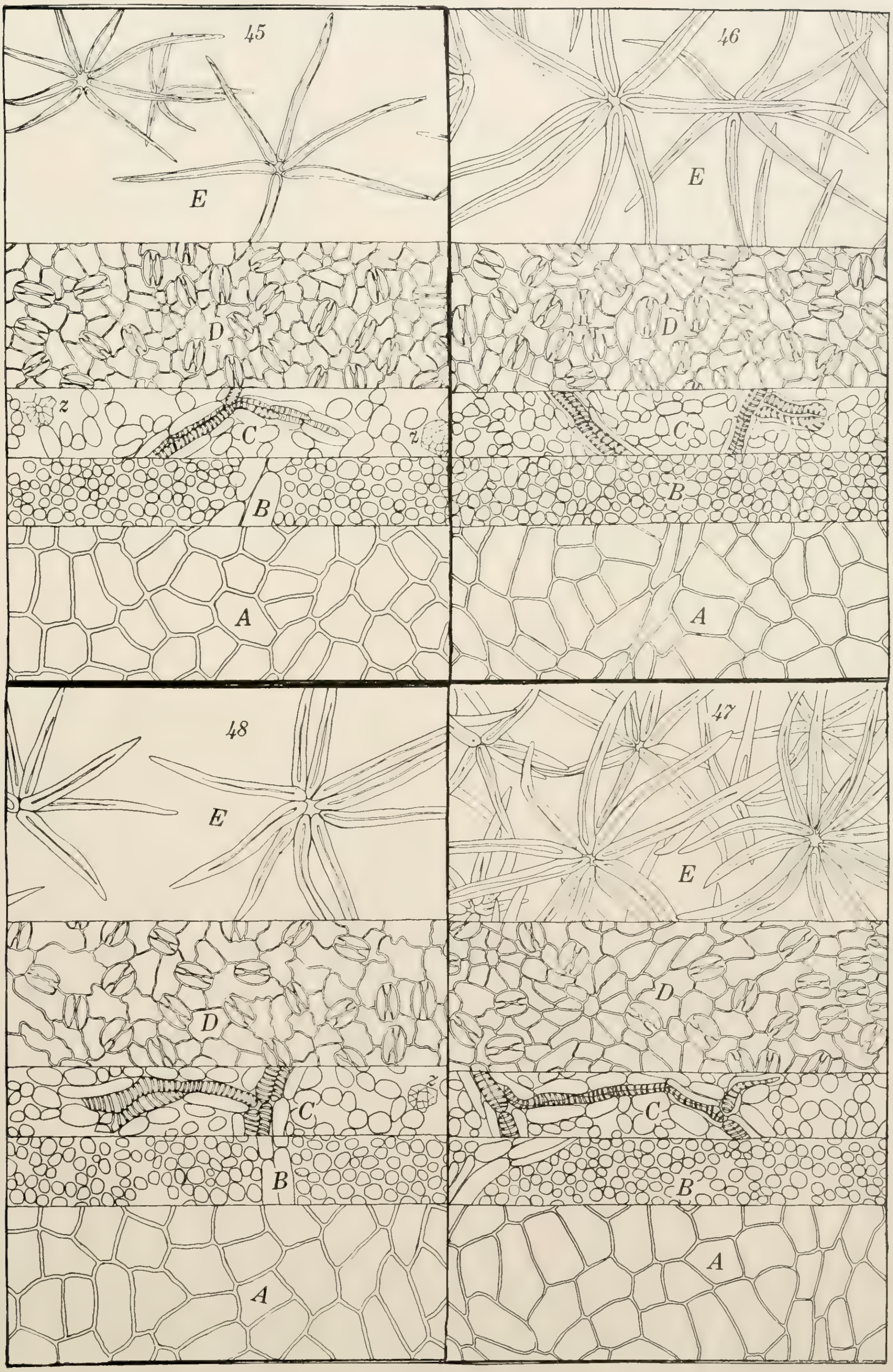
FIG. 45. Tissues of the leaf of No. 1, from bleached leaf. A, upper epidermis; B, palisade cells; C, spongy parenchyma; D, lower epidermis with stomata; E, epidermal hairs; z, crystal of calcium oxylate. × 275.

FIG. 46. Tissues of the leaf of No. 2, from bleached leaf. A, B, C, D, and E, same as in fig. 45. × 275.

FIG. 47. Tissues of the leaf of No. 3, from bleached leaf. A, B, C, D, and E, same as in fig. 45. × 275.

FIG. 48. Tissues of the leaf of No. 4, from bleached leaf. A, B, C, D, and E, same as in fig. 45. × 275.

PLATE XI.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D
z
C
z
B
A
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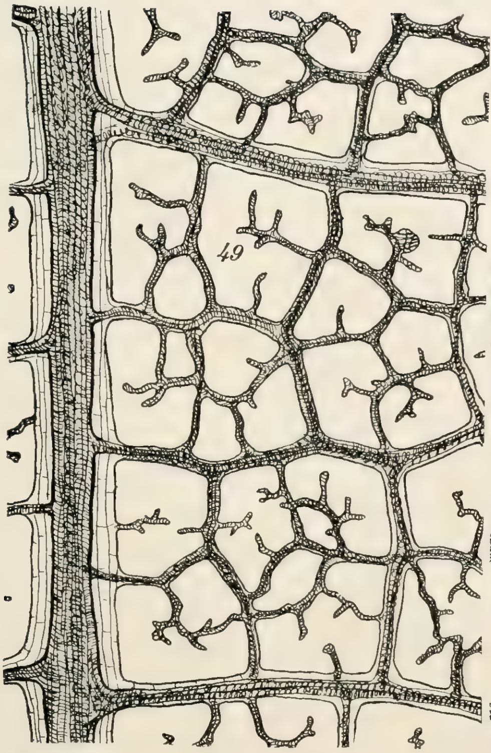
C
B
A
48
E
D
C
z
B
A
47
E
D
C
B
A

PLATE XII.

Fig. 49. Venation of leaf of No. 1. × 55.
Fig. 50. Venation of leaf of No. 2. × 55.
Fig. 51. Venation of leaf of No. 3. × 55.
Fig. 52. Venation of leaf of No. 4. × 55.

PLATE XII.



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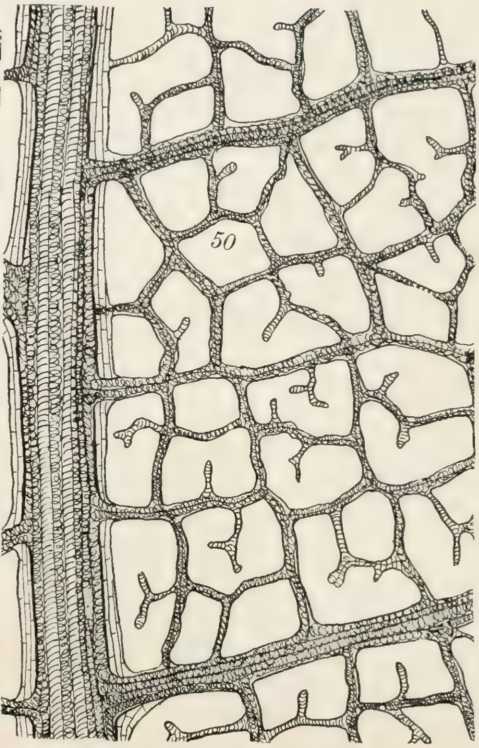

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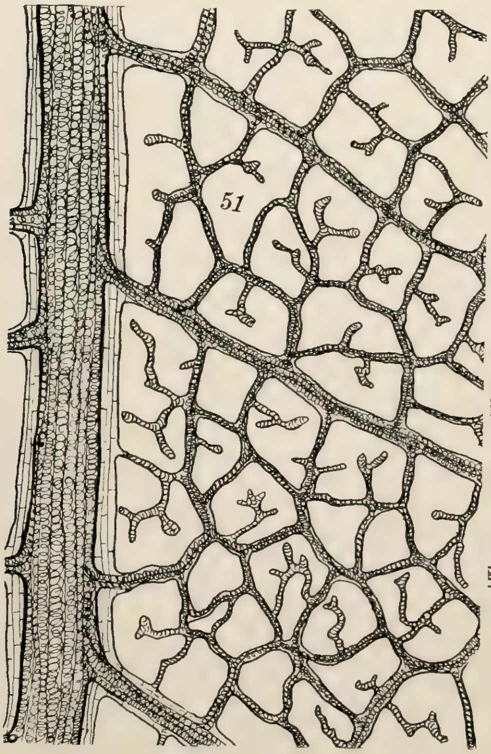

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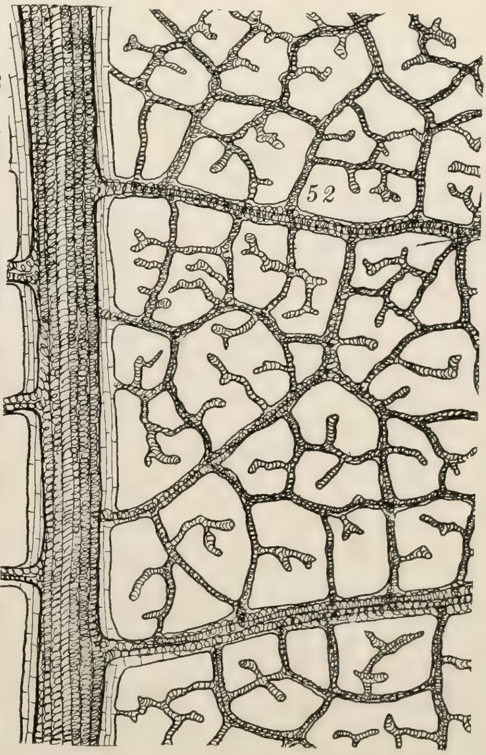

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PLATE XIII.

FIG. 53. Cross section of stem of No. 1, current year; v, pith; w, xylem; x, pholem; y, bast. × 23.

FIG. 54. Cross section of stem of No. 2, current year; v, w, x, y, same as in Fig. 53. × 23.

FIG. 55. Cross section of stem of No. 3, current year; v, w, x, y, same as in Fig. 53. × 23.

FIG. 56. Cross section of stem of No. 4, current year; v, w, x, y, same as in Fig. 53. × 23.

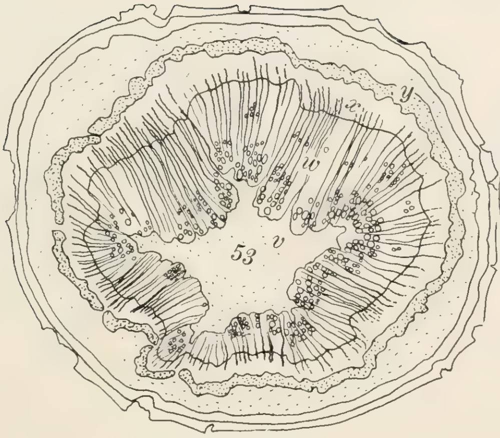
FIG. 57. Tangential section of wood, showing size and frequency of medullary rays, No. 1. × 53.

FIG. 58. Tangential section of wood, No. 2. × 53.

FIG. 59. Tangential section of wood, No. 3. × 53.

FIG. 60. Tangential section of wood, No. 4. × 53.

PLATE XIII.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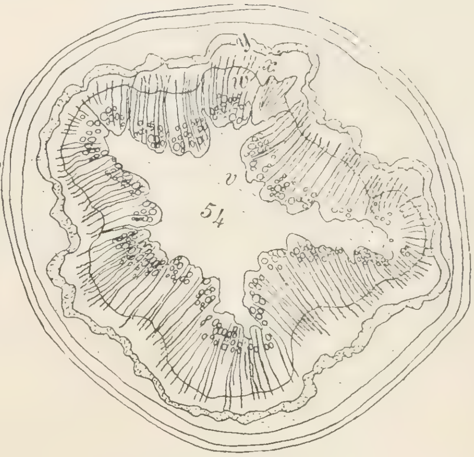
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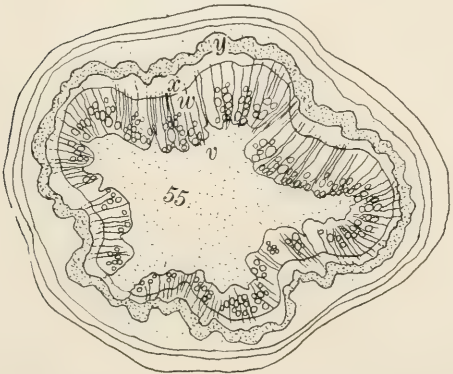
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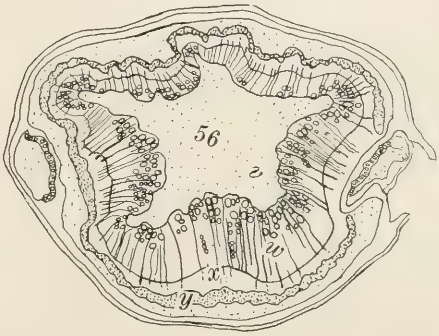
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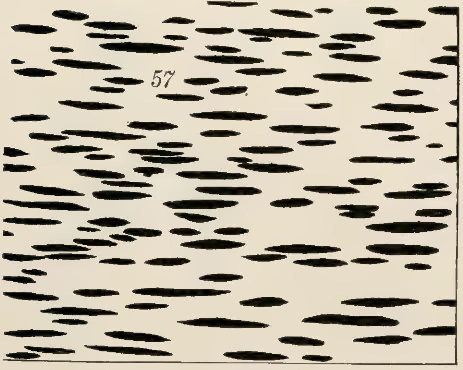
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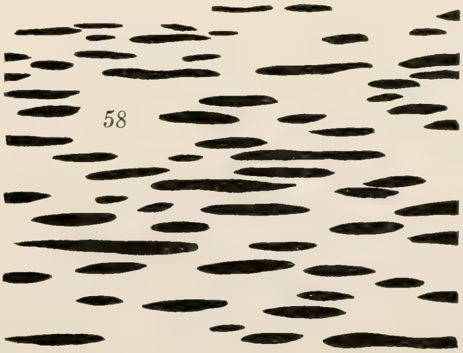
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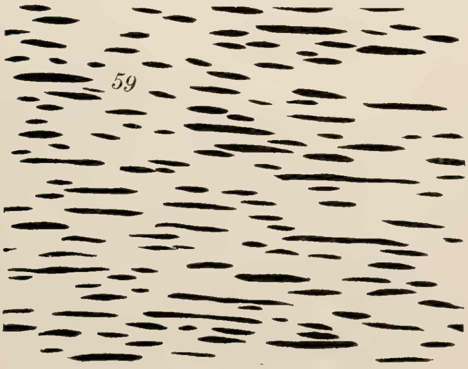
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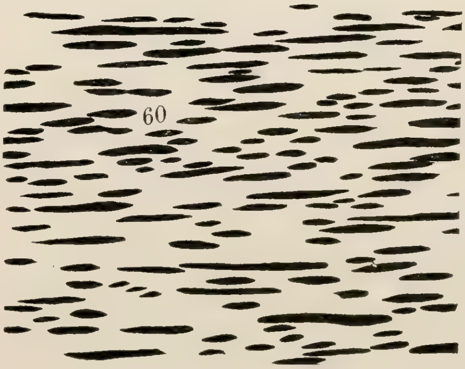
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PLATE XIV.

FIG. 61. Cross section of stem, No. 1, in sixth year, showing rings of growth, tracheal tubes, medullary rays, and bark. Bast stipuled. × 23.

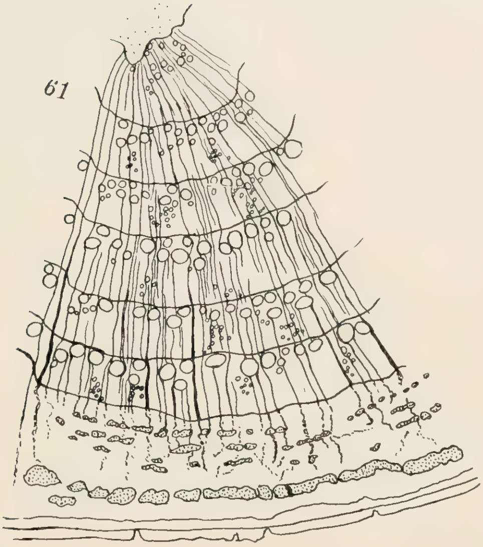
FIG. 62. Cross section of stem, No. 2, in seventh year, showing same elements as fig. 61. × 23.

FIG. 63. Cross section of stem, No. 3, in fourth year, showing same elements as fig. 61. × 23.

FIG. 64. Cross section of stem, No. 4, in third year, showing same elements as fig. 61. × 23.

PLATE XIV.

61



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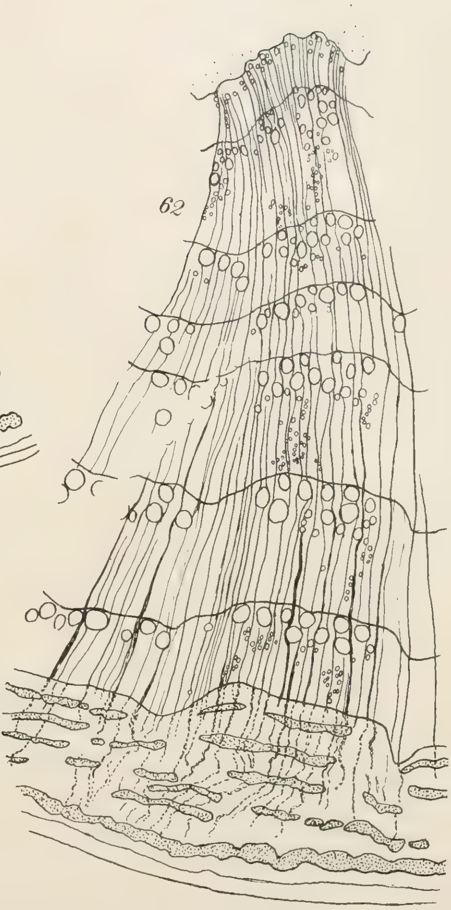

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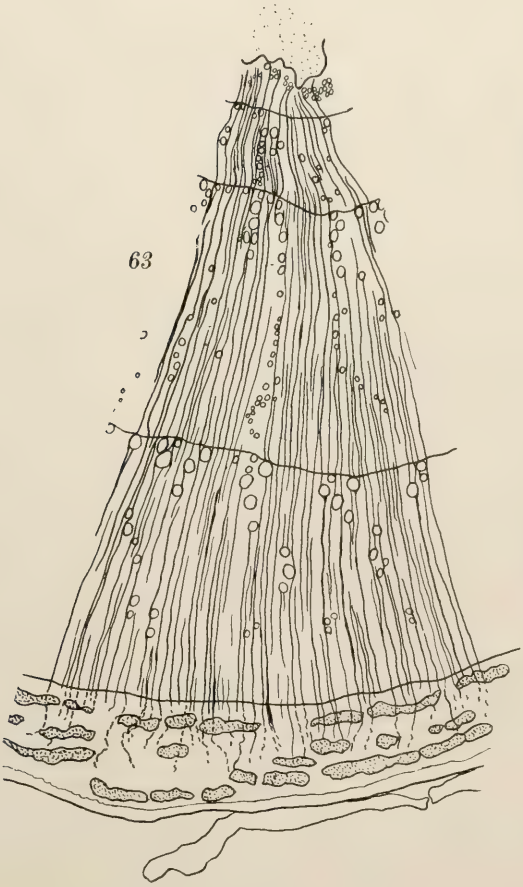

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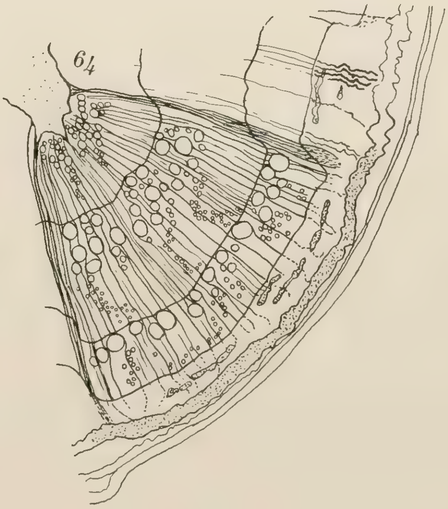


PLATE XV.

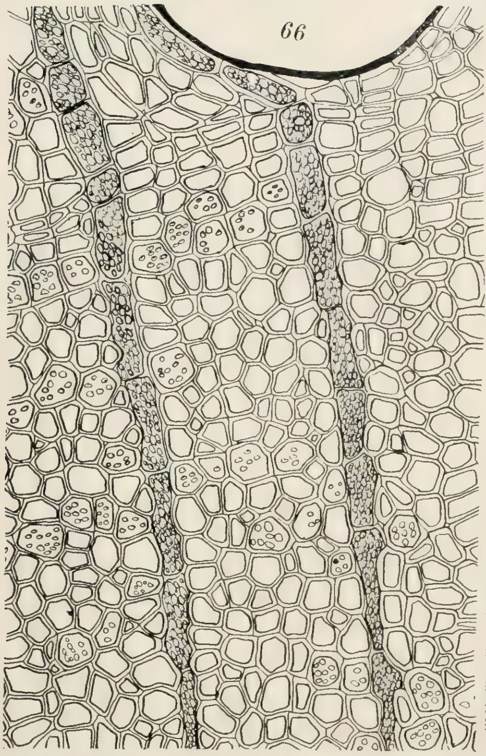
FIG. 65. Cross section of wood, No. 1, in fourth year, showing wood fiber, medullary rays, and part of cross section of tracheal tube. (Heavy wall.) × 275.

FIG. 66. Cross section of wood, No. 2, in fourth year. × 275.

FIG. 67. Cross section of wood, No. 3, in fourth year. × 275.

FIG. 68. Cross section of wood, No. 4, in third year. × 275.

PLATE XV.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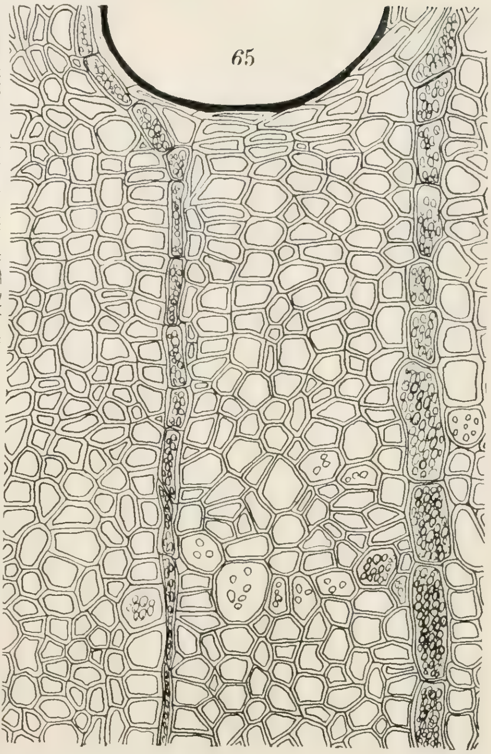
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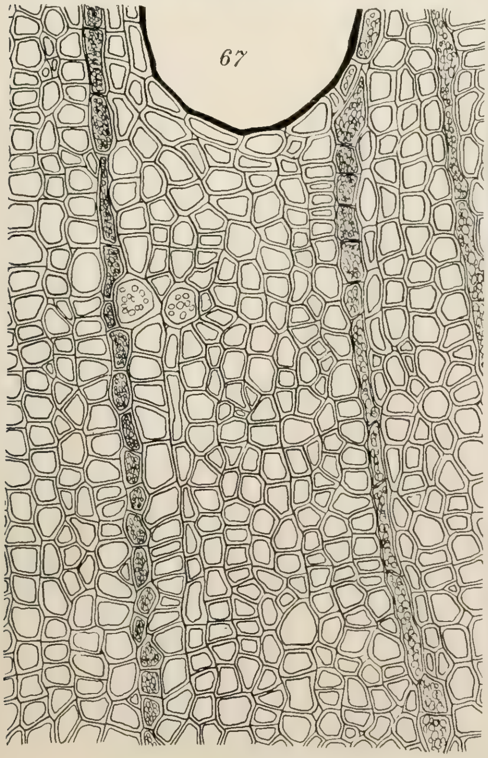
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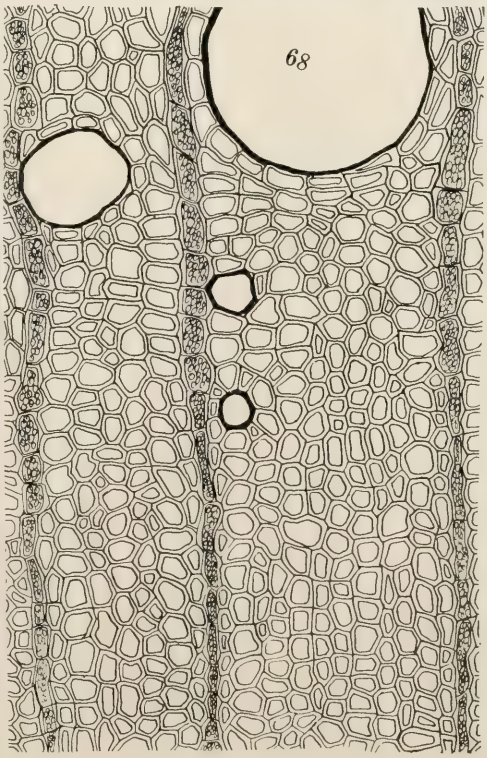
68

PLATE XVI.

FIG. 1. Tangential section of leaf of X. *pennsylvanicum.* × 200. *A*, border parenchyma cells; *B*, tracheal vessels; *C*, photosynthetic tissue.

FIG. 2. Tangential section of leaf of X. *globosum.* × 200. *D*, border parenchyma cells; *E*, tracheal vessels, *F*, photosynthetic tissue.

FIG. 3. Tangential section of leaf of X. *americanum.* × 200. *C*, border parenchyma cells; *H*, tracheal vessels; *I*, photosynthetic tissue.

FIG. 4. Cross section of leaf of X. *americanum.* × 193. *J*, epidermis; *K*, tracheal vessels; *L*, border parenchyma cells; *M*, photosynthetic cells; *N*, vascular bundle; *O*, trichome.

FIG. 5. Photosynthetic cells from the leaf of X. *pennsylvanicum.* × 193.

FIG. 6. Smaller photosynthetic cells from the leaf of X. *pennsylvanicum.* × 193.

FIG. 7. Photosynthetic cells from the leaf of X. *Globosum.* × 193.

FIG. 8. Photosynthetic cells from the leaf of X. *americanum.* × 193.

FIG. 9. Cross section of leaf of X. *pennsylvanicum.* × 193. *P*, stoma; *Q*, epidermis; *R*, vessels; *S*, water-storage cells; *T*, vascular bundle; *U*, trichome from leaf.

PLATE XVI.

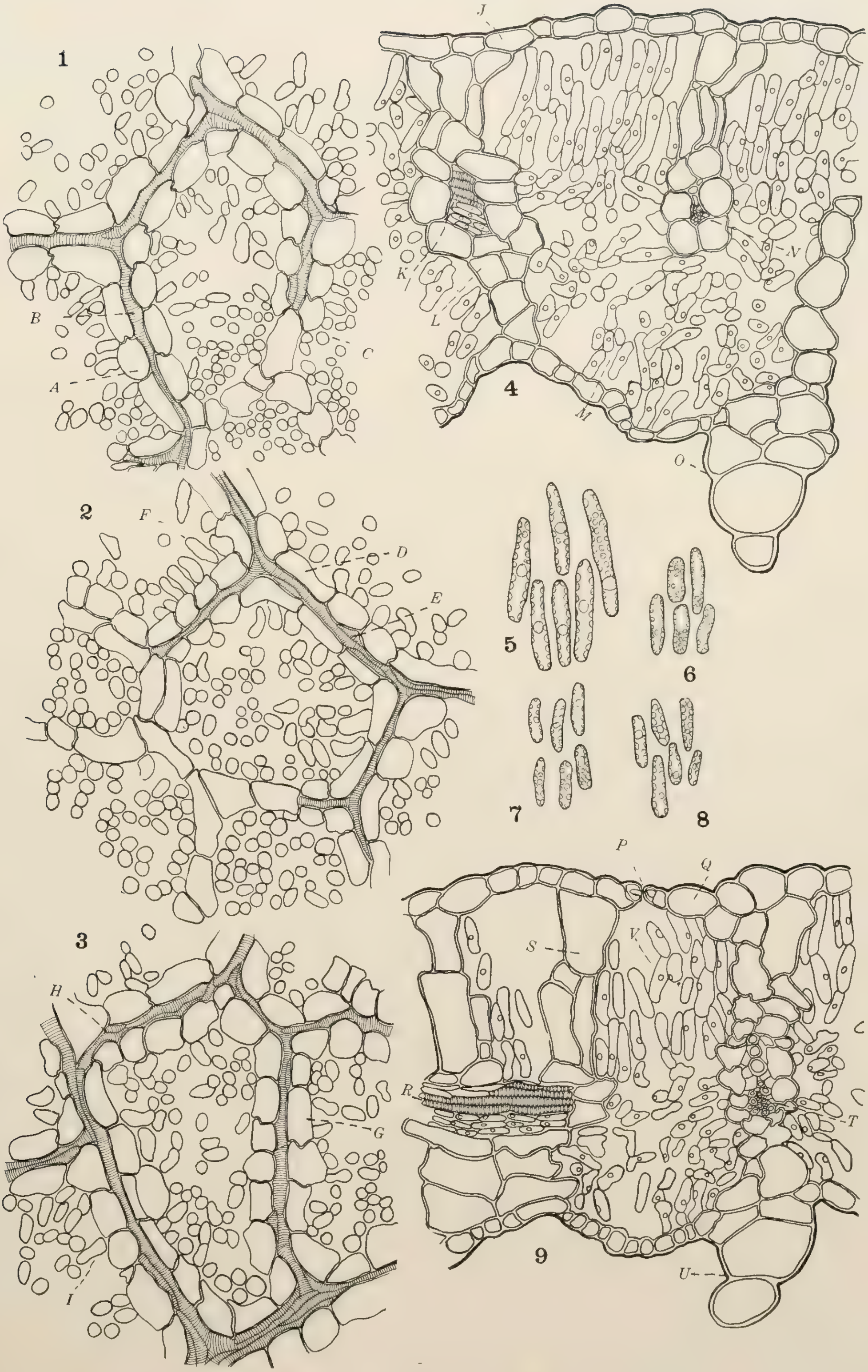
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PLATE XVII.

FIG. 10. Apex of bleached leaf of *X. globosum* showing serration. × 11. *W*, clusters of tracheids; *X*, hairs from leaf.

FIG. 11. Cross section of midrib of leaf of *X. globosum.* × 27. *A*, photosynthetic tissue; *B*, water-storage cells; *C*, collenchyma of midrib; *D*, epidermis; *X*, xylem of vascular bundle; *Y*, phloëm.

FIG. 12. Cross section at the base of leaf of *X. pennsylvanicum.* × 17. *E*, vasular bundle; *F*, collenchyma; *G*, resin duct; *H*, photosynthetic cells; *I*, water-storage cells; *J*, midrib of leaf.

FIG. 13. Tracheids from leaf of *X. globosum.* × 193.

FIG. 14. Stomata from leaf of *X. globosum.* × 193. *K*, epidermal cells; *L*, photosynthetic cells; *M*, stoma.

FIG. 15. Epidermis of midrib of leaf of *X. pennsylvanicum.* × 200. *N*, epidermis.

FIG. 16. Tangential section of epidermis from the bleached leaf of *X. globosum.* × 200. *O*, stoma.

FIG. 17. Tangential section of epidermis of the leaf of *X. americanum.* × 193. *Q*, cells at base of hairs; *R*, stoma; *S*, epidermal cells.

FIG. 18. Trichomes on leaf of *X. americanum.* × 193. *T*, cells at base of hairs.

FIG. 19. Tangential section from bleached leaf of *X. pennsylvanicum.* × 200. *U*, stoma.

FIG. 20. Tangential section from bleached leaf of *X. americanum.* × 200. *V*, stoma.

FIG. 21. Central vascular bundle from leaf of *X. pennsylvanicum,* × 127. *W*, vessels; *X*, phloëm.

FIG. 22. Composite drawing of leaf of *X. globosum.* × 193. *Y*, vessels; *Z*, border parenchyma; *A*, epidermal cells; *B*, cells at base of hairs; *C*, stoma; *D*, basal cell of hair; *E*, linear hair; *F*, pointed hairs.

PLATE XVII.

FIG. 10. Apex of bleached leaf of X. *globosum* showing serration. × 11. *W*, clusters of tracheids; *X*, hairs from leaf.

FIG. 11. Cross section of midrib of leaf of X. *globosum*. × 27. *A*, photosynthetic tissue; *B*, water-storage cells; *C*, collenchyma of midrib; *D*, epidermis; *X*, xylem of vascular bundle; *Y*, phloëm.

FIG. 12. Cross section at the base of leaf of X. *pennsylvanicum*. × 17. *E*, vasular bundle; *F*, collenchyma; *G*, resin duct; *H*, photosynthetic cells; *I*, water-storage cells; *J*, midrib of leaf.

FIG. 13. Tracheids from leaf of X. *globosum*. × 193.

FIG. 14. Stomata from leaf of X. *globosum*. × 193. *K*, epidermal cells; *L*, photosynthetic cells; *M*, stoma.

FIG. 15. Epidermis of midrib of leaf of X. *pennsylvanicum*. × 200. *N*, epidermis.

FIG. 16. Tangential section of epidermis from the bleached leaf of X. *globosum*. × 200. *O*, stoma.

FIG. 17. Tangential section of epidermis of the leaf of X. *americanum*. × 193. *Q*, cells at base of hairs; *R*, stoma; *S*, epidermal cells.

FIG. 18. Trichomes on leaf of X. *americanum*. × 193. *T*, cells at base of hairs.

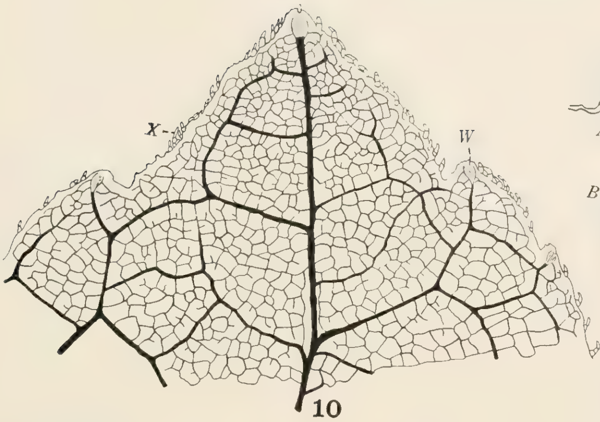
FIG. 19. Tangential section from bleached leaf of X. *pennsylvanicum*. × 200. *U*, stoma.

FIG. 20. Tangential section from bleached leaf of X. *americanum*. × 200. *V*, stoma.

FIG. 21. Central vascular bundle from leaf of X. *pennsylvanicum*, × 127. *W*, vessels; *X*, phloëm.

FIG. 22. Composite drawing of leaf of X. *globosum*. × 193. *Y*, vessels; *Z*, border parenchyma; *A*, epidermal cells; *B*, cells at base of hairs; *C*, stoma; *D*, basal cell of hair; *E*, linear hair; *F*, pointed hairs.

PLATE XVII.



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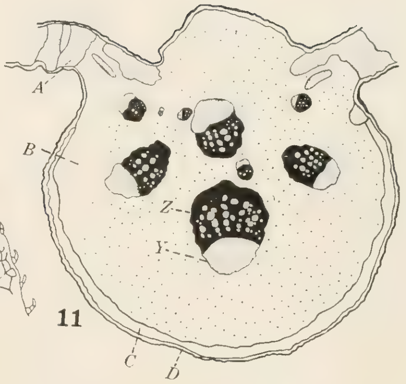

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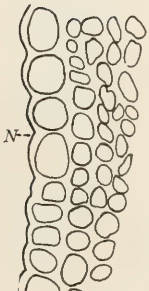

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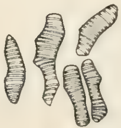

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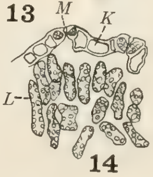

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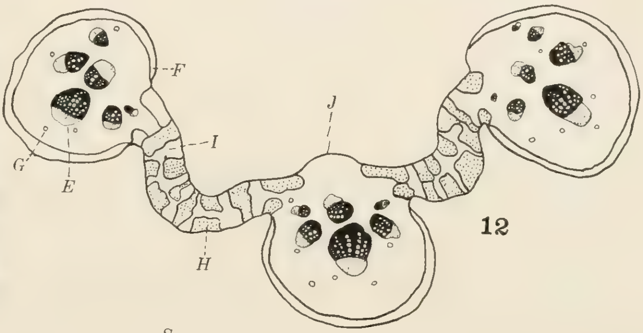

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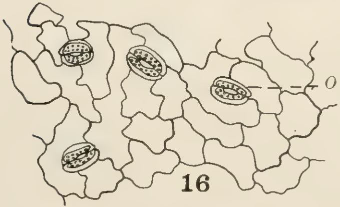

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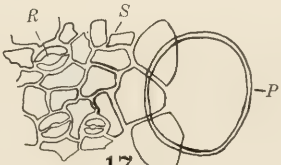

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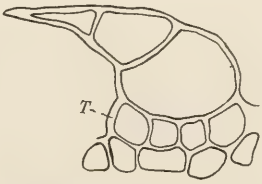

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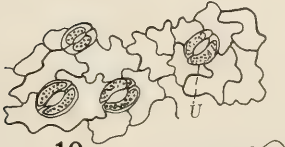

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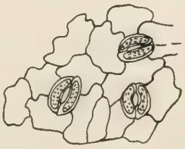

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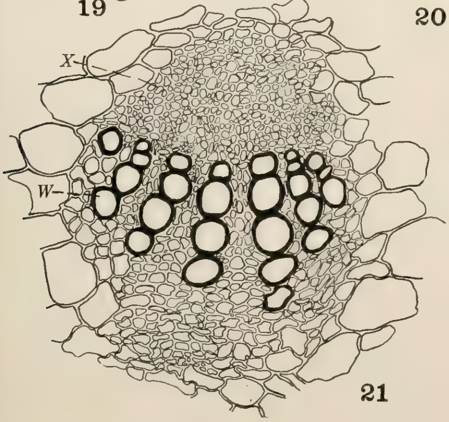

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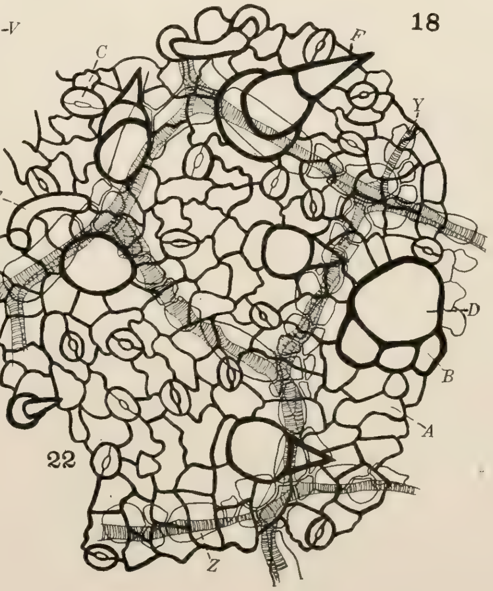

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PLATE XVIII.

FIG. 23. Tangential section of bleached leaf of *X. globosum,* showing relation of trichomes to veins. × 43. *G,* veins; *H,* trichome.

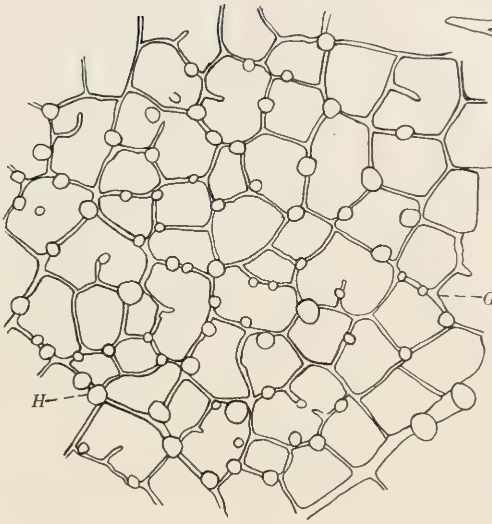
FIG. 24. Large trichomes from leaf of *X. americanum.* × 193. *I,* cells at base of hairs; *J,* stoma; *K,* epidermal cells; *L,* basal cell of hairs.

FIG. 25. Diagram of cross section of stem of *X. americanum.* × 11. *M,* collenchyma; *N,* resin duct; *O,* bast; *P,* phloëm; *Q,* xylem; *R,* pith; *S,* parenchyma of pericycle.

FIG. 26. Diagram of cross section of stem of *X. pennsylvanicum.* × 11. *T,* collenchyma; *U,* parenchyma of pericycle; *V,* resin duct; *W,* bast, *X,* phloëm; *Y,* xylem; *Z,* pith.

FIG. 27. Diagram of cross section of stem of *X. globosum.* × 11. *A,* epidermis; *B,* collenchyma; *C,* parenchyma of pericycle; *D,* resin duct; *E,* bast; *F,* phloëm, *G,* xylem.

PLATE XVIII.



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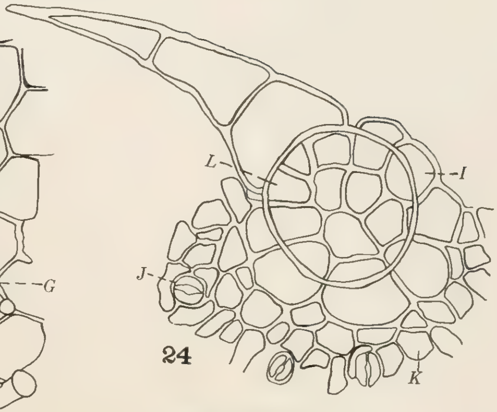

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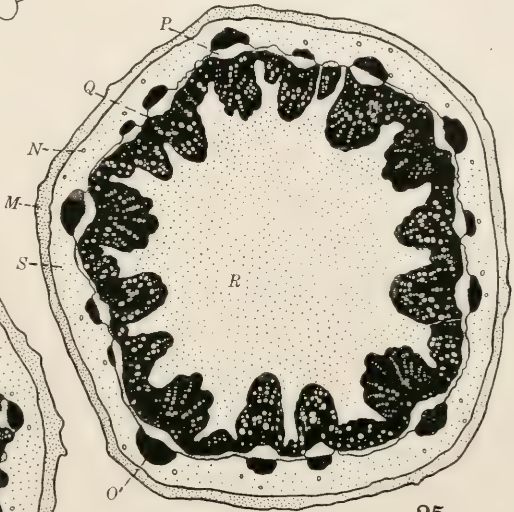

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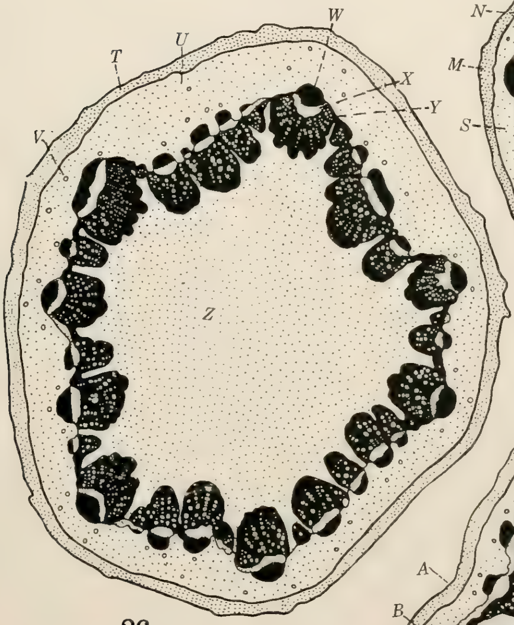

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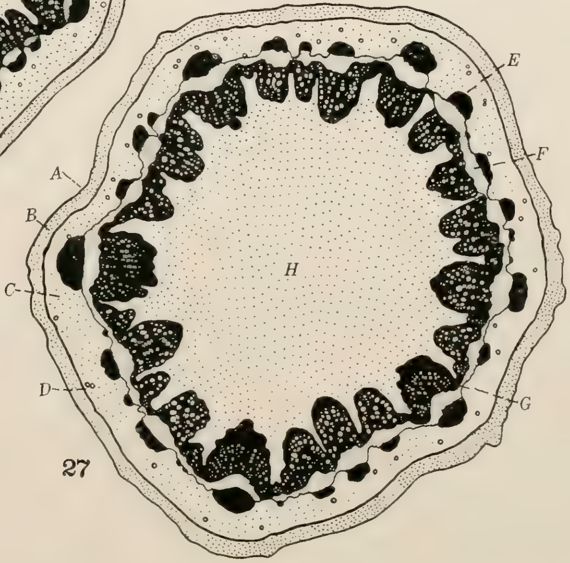


27

PLATE XIX.

FIG. 28. Large vascular bundle at angle of stem of X. *globosum.* × 193. *I,* tracheal tube; *J,* parenchyma; *K,* bast; *L,* phloëm; *M,* wood parenchyma.

FIG. 29. Cross section of pith of stem of *X. pennsylvanicum.* × 133.

FIG. 30. Cross section of pith of stem of *X. americanum.* × 133.

FIG. 31. Cross section of pith of stem of *X. globosum.* × 133.

FIG. 32. Cross section of pith of *X. pennsylvanicum,* showing calcium oxalate crystals. × 193. *N,* calcium oxalate crystals.

FIG. 33. Longitudinal section of pith of *X. globosum.* × 193. *O,* calcium oxalate crystals.

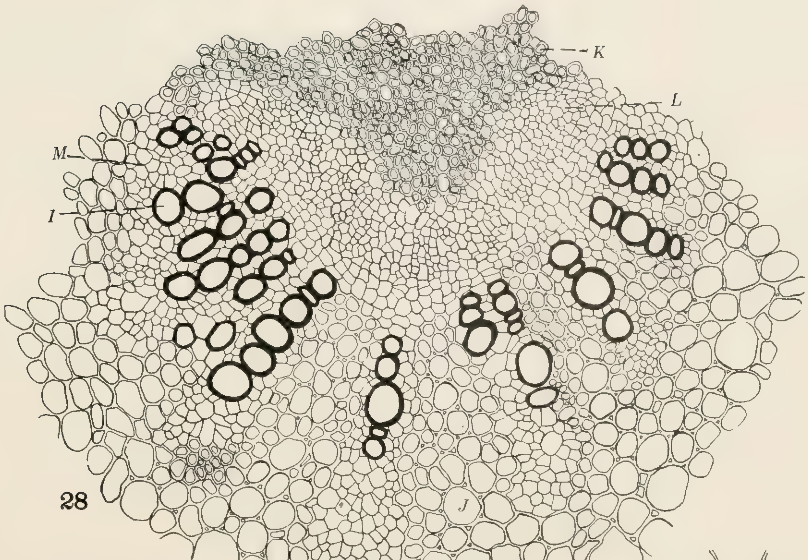
FIG. 34. Cross section of phloëm from the stem of *X. pennsylvanicum.* × 133.

FIG. 35. Cross section of starch sheath from stem of *X. globosum.* × 193. *P,* calcium oxalate crystals; *Q,* starch grains; *R,* starch sheath.

FIG. 36. Cross section of starch sheath from the stem of *X. americanum.* × 193. *S,* starch sheath; *T,* starch grains; *U,* calcium oxalate crystals.

FIG. 37. Cross section of the starch sheath from the stem of *X. globosum.* × 193. *V,* starch sheath; *W,* starch grains; *X,* calcium oxalate crystals.

PLATE XIX.



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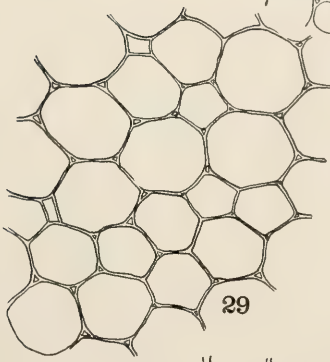

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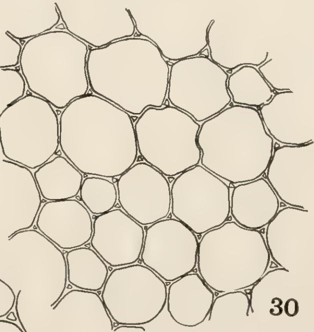

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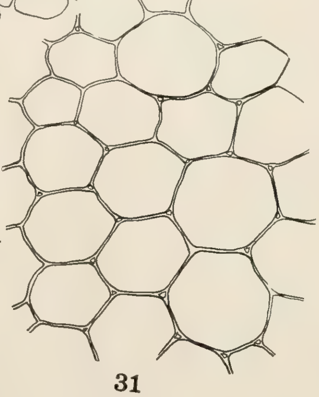

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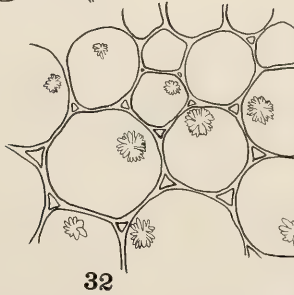

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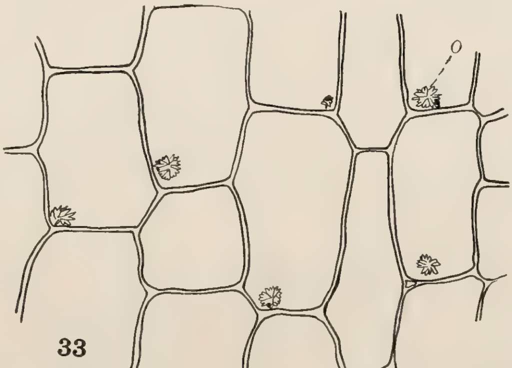

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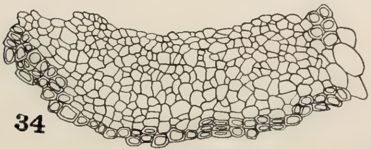

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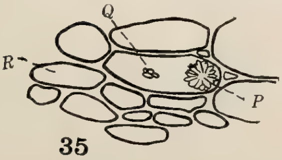

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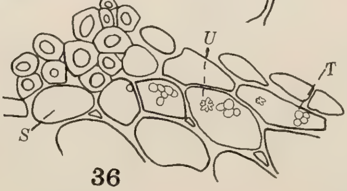

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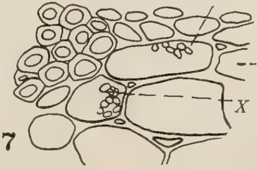

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PLATE XX.

Fig. 38. Vascular bundle from the stem of *X. pennsylvanicum.* × 140. *A*, wood fibers; *B*, phloëm; *C*, bast; *Y*, tracheal vessels; *Z*, wood parenchyma.

Fig. 39. Vascular bundle from the stem of *X. americanum.* ×133. *D*, tracheal vessel; *E*, wood fibers; *F*, wood parenchyma; *G*, phloëm; *H*, bast.

Fig. 40. Vascular bundle from the stem of *X. globosum.* × 140. *I*, tracheal vessels; *J*, wood parenchyma; *K*, wood fibers; *L*, phloëm; *M*, bast.

Fig. 41. Cross section of portion of two bundles of stem of *X. globosum.* × 43. *N*, elongated ray cells.

Fig. 42. Cross section of base of bundle of *X. americanum.* × 193. *O*, tracheal vessel; *P*, lignified cells.

Fig. 43. Longitudinal section of wood parenchyma from *X. globosum.* × 193.

Fig. 44. Wood fibers from *X. globosum.* × 193.

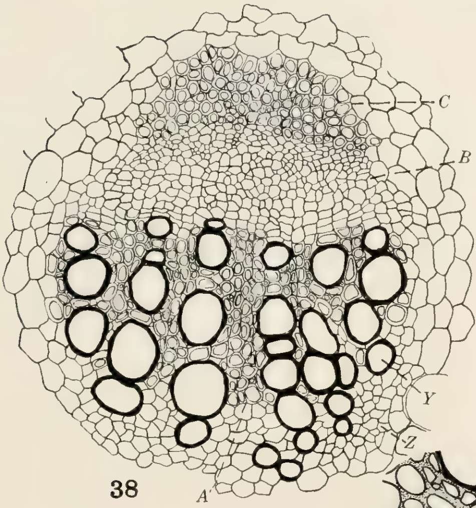
Fig. 45. Tracheal elements in stem of *X. pennsylvanicum.* × 200.

Fig. 46. Tracheal elements in stem of *X. globosum.* × 200.

Fig. 47. Tracheal elements in stem of *X. americanum.* × 200.

Fig. 48. Wood fibers from *X. americanum.* × 193.

PLATE XX.



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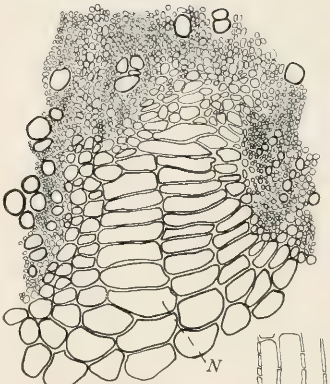

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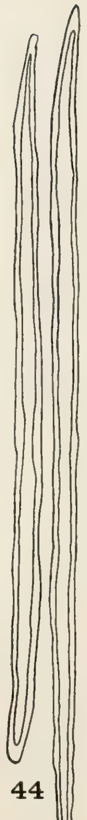

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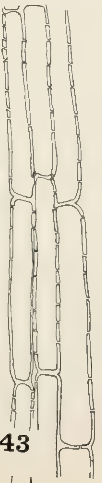

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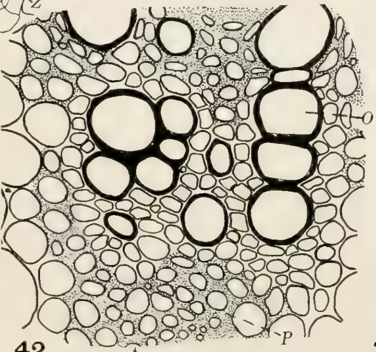

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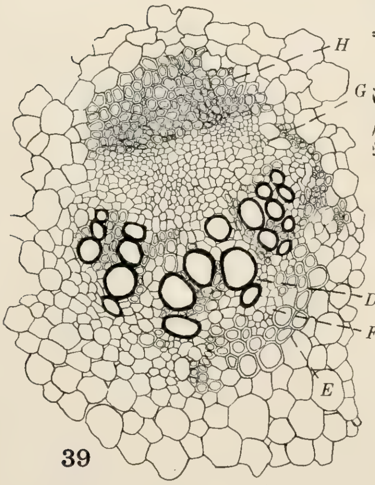

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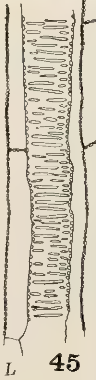

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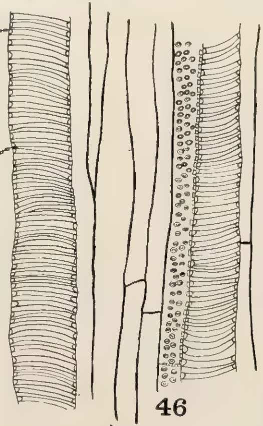

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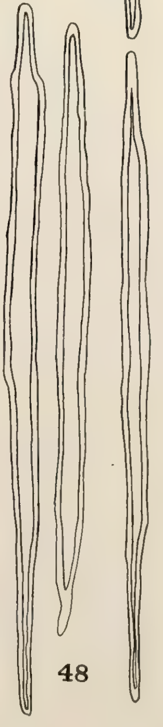

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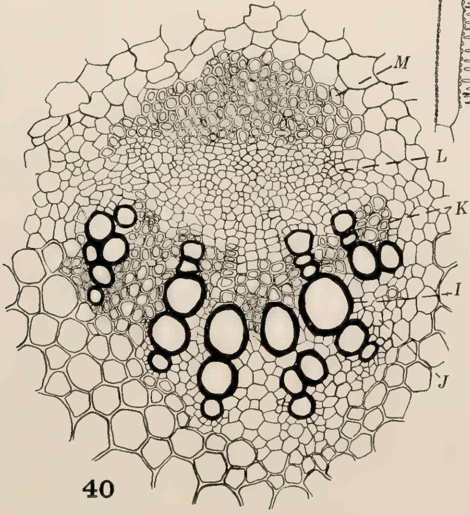

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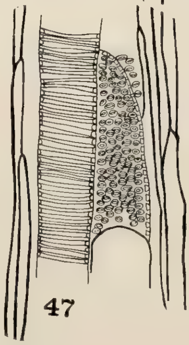

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PLATE XXI.

FIG. 49. Cross section of portion of cortex of *X. pennsylvanicum.* × 200. *Q*, epidermis; *R*, collenchyma; *S*, parenchyma of pericycle; *T*, resin duct.

FIG. 50. Cross section of portion of cortex of *X. globosum.* × 200. *U*, epidermis; *V*, collenchyma; *W*, parenchyma of pericycle; *X*, resin duct.

FIG. 51. Longitudinal section of parenchyma of pericycle of *X. globosum.* × 193.

FIG. 52. Cross section of parenchyma of pericycle of *X. globosum.* × 193.

FIG. 53. Cross section of parenchyma of pericycle of *X. globosum*, showing resin duct. × 193. *Y*, parenchyma; *L*, resin duct.

FIG. 54. Trichome from surface of stem of *X. globosum.* × 193. *A*, epidermis; *B*, trichome.

FIG. 55. Tangential section of epidermis of stem of *X. globosum.* × 193. *C*, epidermal cell; *D*, glucosides.

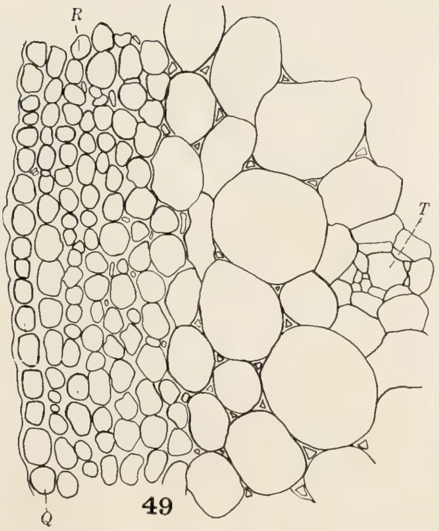
FIG. 56. Tangential section of epidermis of stem of *X. americanum.* × 193. *E*, epidermis; *F*, glucosides.

FIG. 57. Cross section of epidermis of *X. americanum.* × 193.

FIG. 58. Cross section of epidermis of *X. pennsylvanicum.* × 193.

FIG 59. Longitudinal section of epidermis of stem of *X. pennsylvanicum.* × 193.

PLATE XXI.



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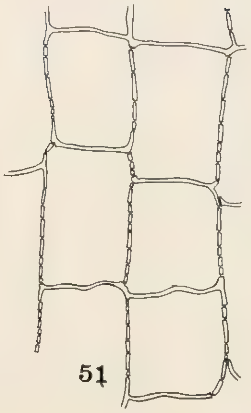

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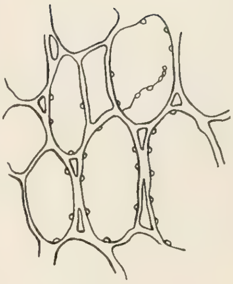

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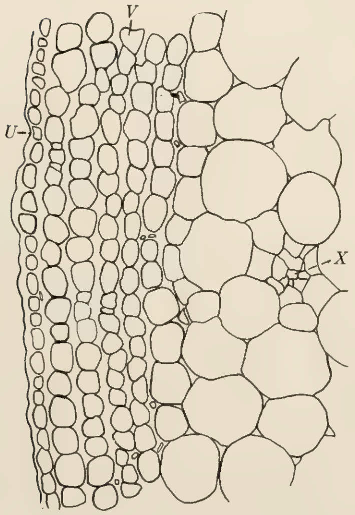

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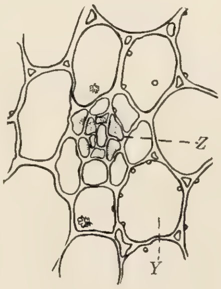

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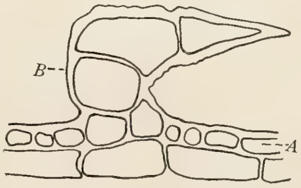

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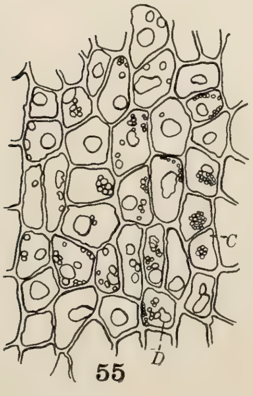

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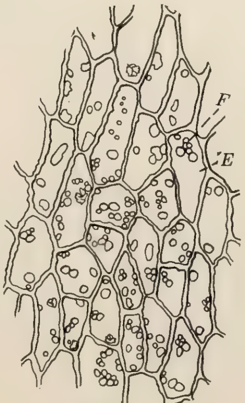

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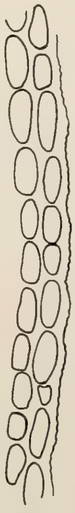

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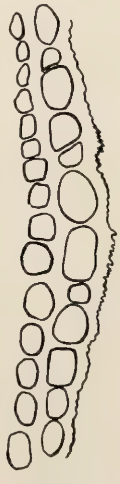

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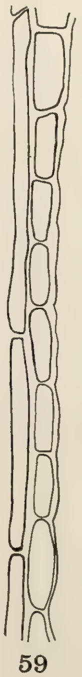

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PLATE XXII.

Fig. 60. Cross section of root of *X. globosum.* × 17. *G*, bark; *H*, phloëm; *I*, resin duct; *J*, medullary ray; *K*, tracheal tube.

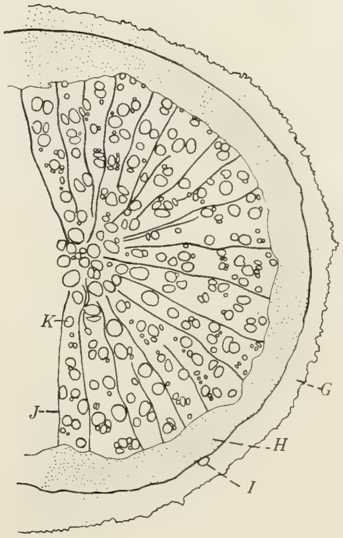
Fig. 61. Cross section of root of *X. pennsylvanicum.* × 17. *L*, bark; *M*, phloëm; *N*, resin duct; *O*, medullary ray; *P*, tracheal tube.

Fig. 62. Cross section of bast of *X. pennsylvanicum.* × 193. *Q*, starch sheath; *R*, bast.

Fig. 63. Cross section of bast of *X. globosum.* × 193. *S*, starch sheath; *T*, bast.

Fig. 64. Longitudinal section of bast of *X. globosum.* × 193.

PLATE XXII.



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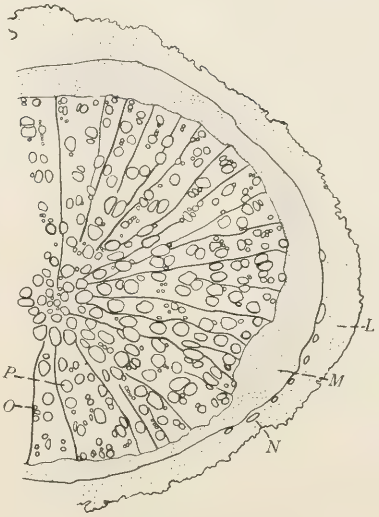

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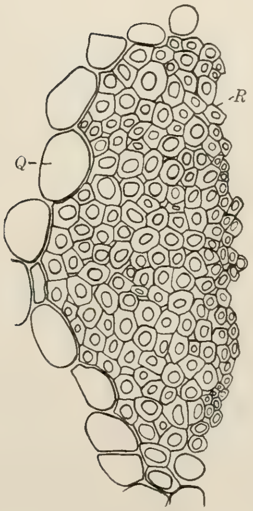

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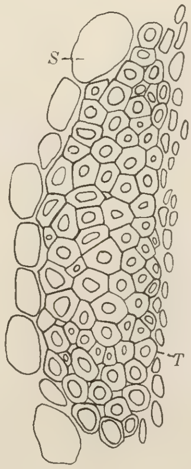

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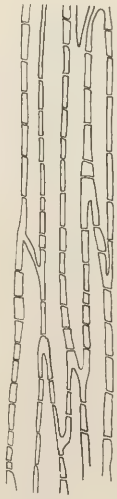

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PLATE XXIII.

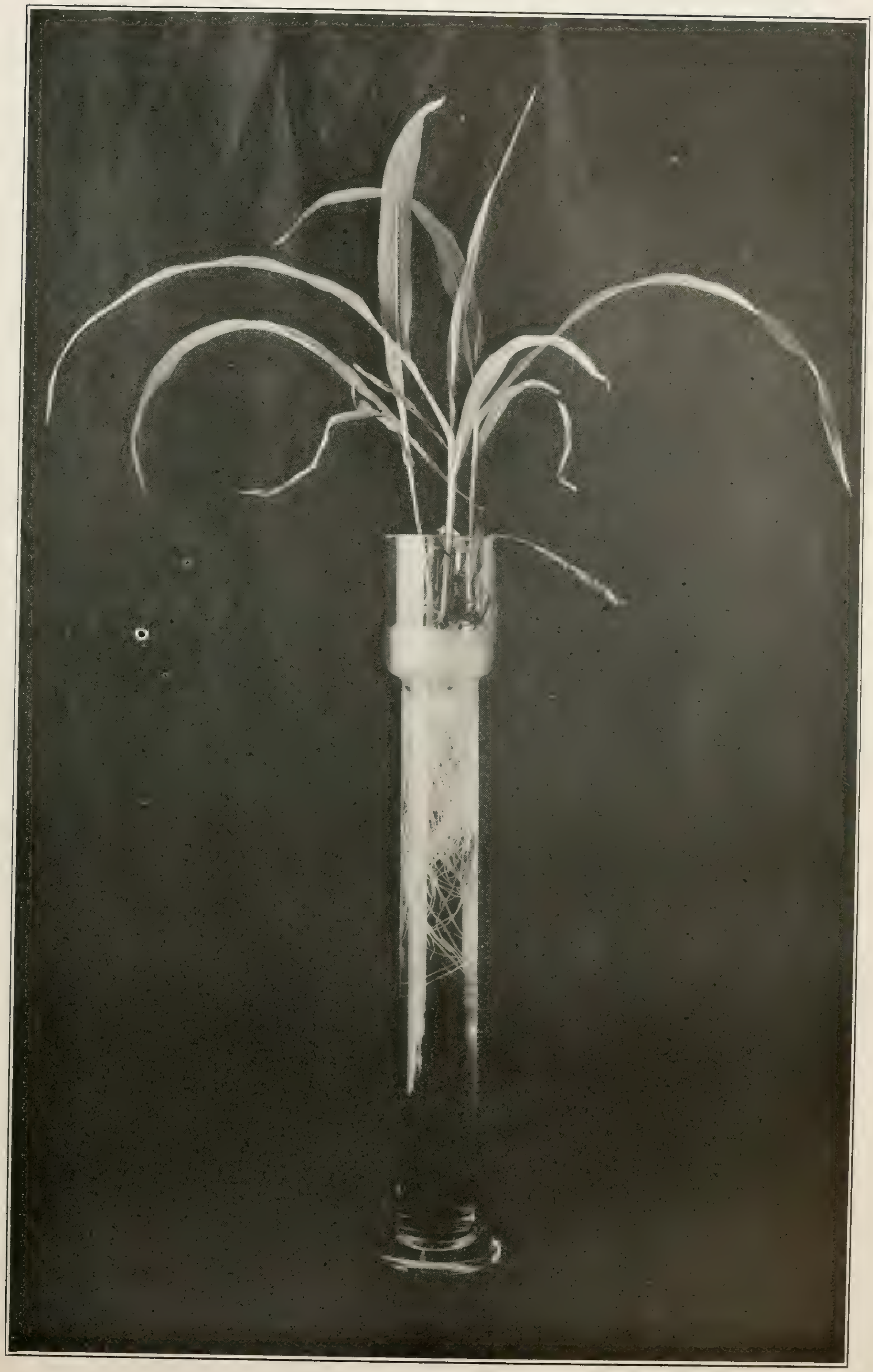


PLATE XXIII. Showing method of supporting the growing seedlings.

PLATE XXIV.

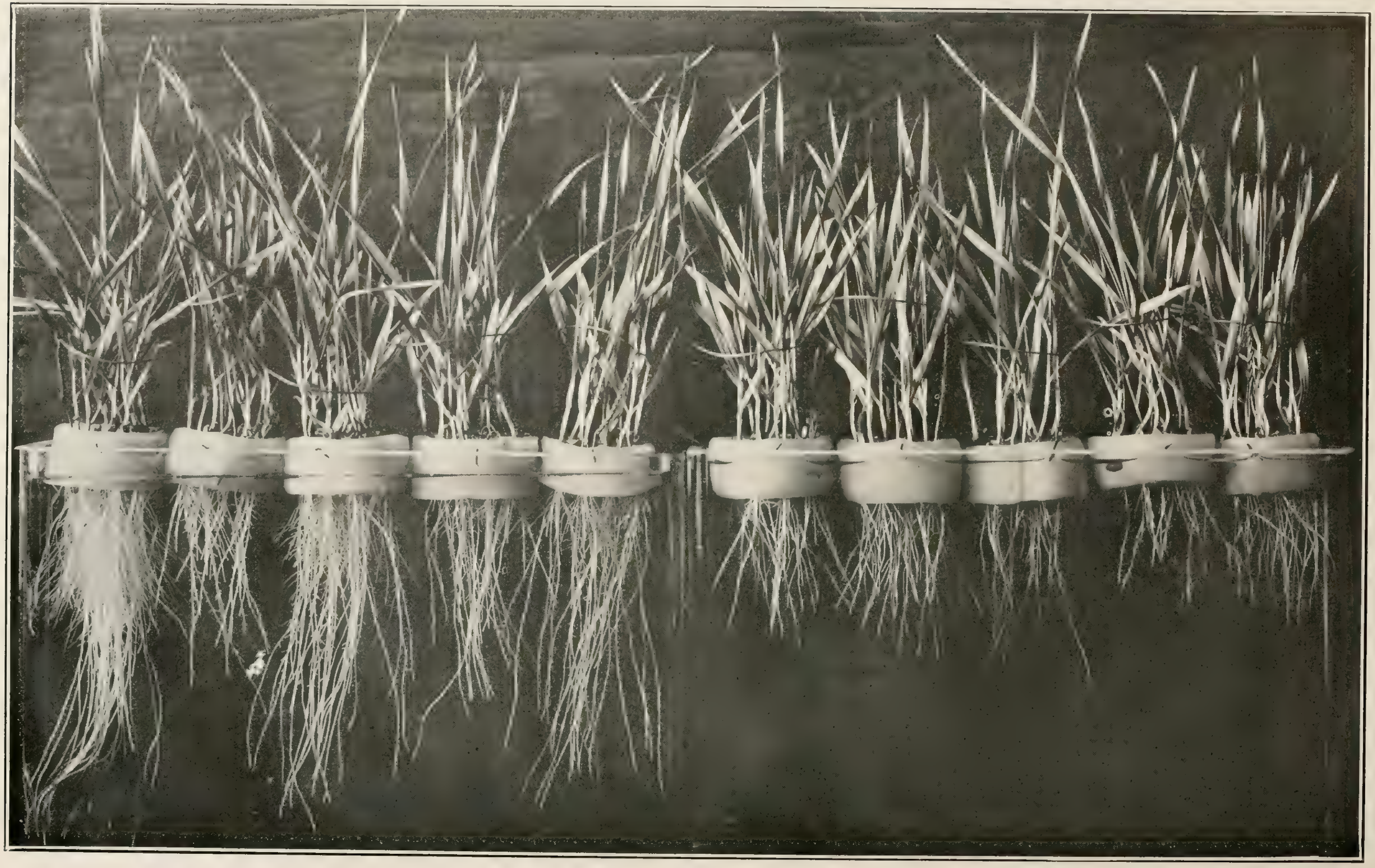
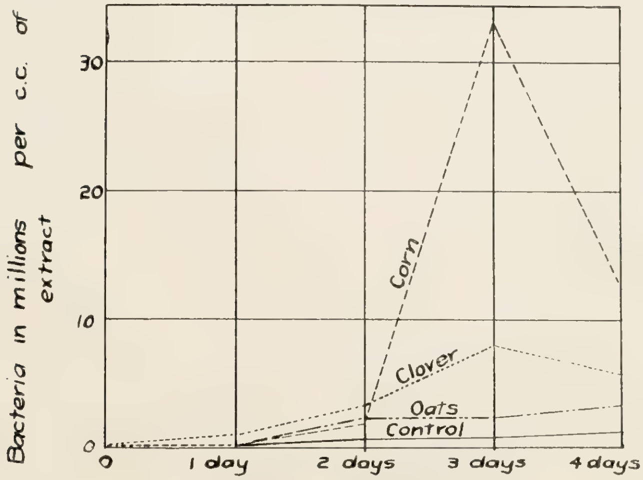
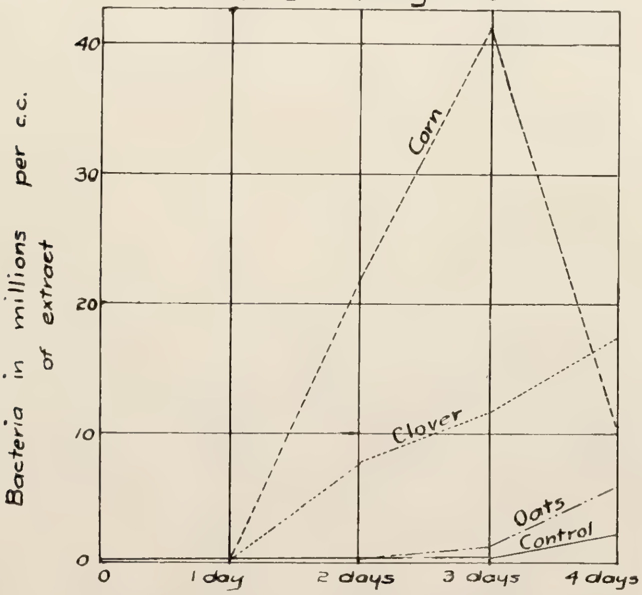


Plate XXIV. Wheat seedlings were grown for 18 days in extracts from soils in which the following kinds of organisms had grown. Reading from left to right: Ammonifier; B. radicicola; Azotobacter; B. prodigiosus; B. liquefaciens fluorescens; Cladothrix odorifera; Aspergillus niger; sterile soil; normal soil flora; B. Subtilis.

PLATE XXV.

Showing rate of development of various organisms in extracts of marsh soil cropped in different ways.


Yellow ammonifier


Bacillus Prodigiosus

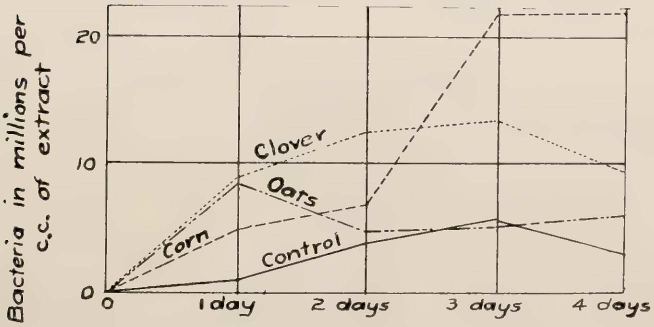

Bacillus proteus vulgaris

PLATE XXVI.

Showing rate of development of various organisms in extracts of loam soil cropped in different ways.

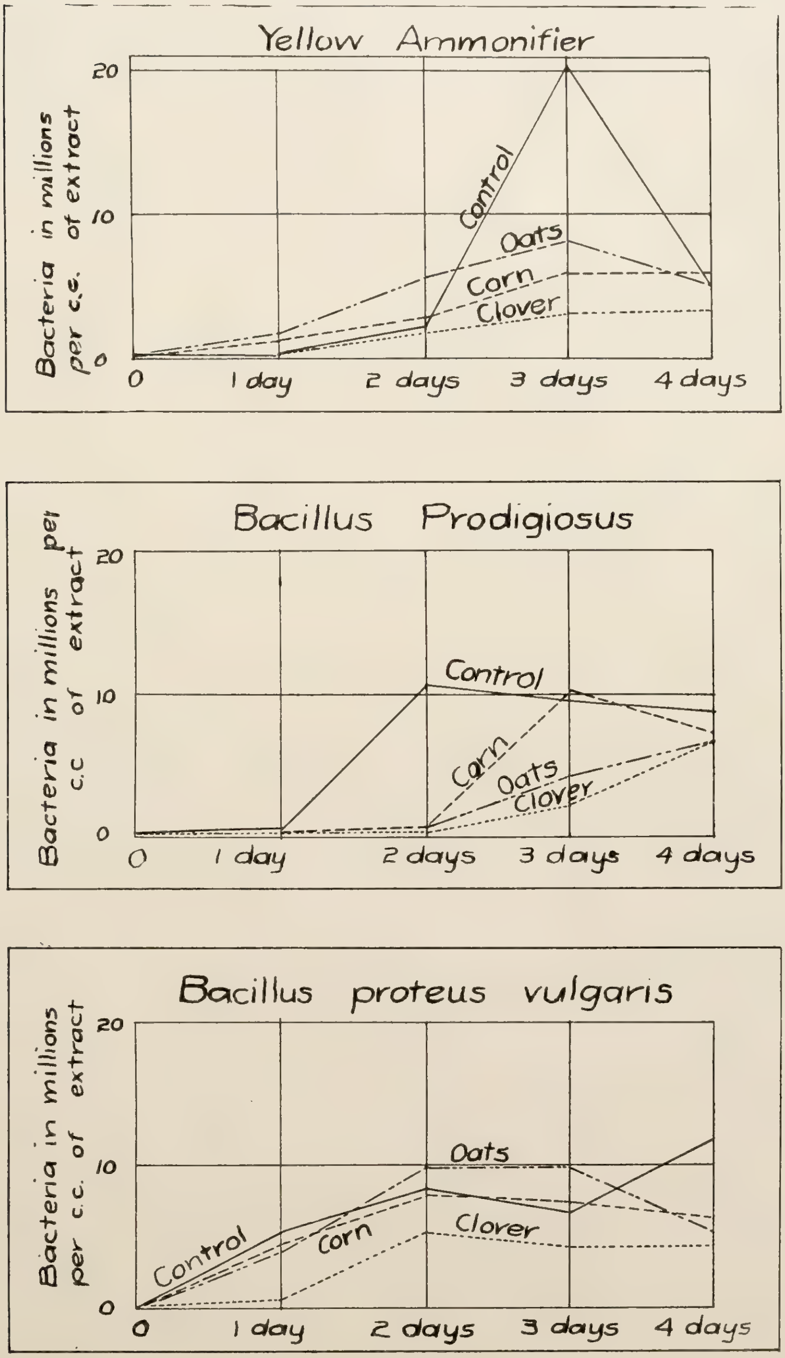


PLATE XXVII.

Showing rate of development of various organisms in extracts of sand soils cropped in different ways.

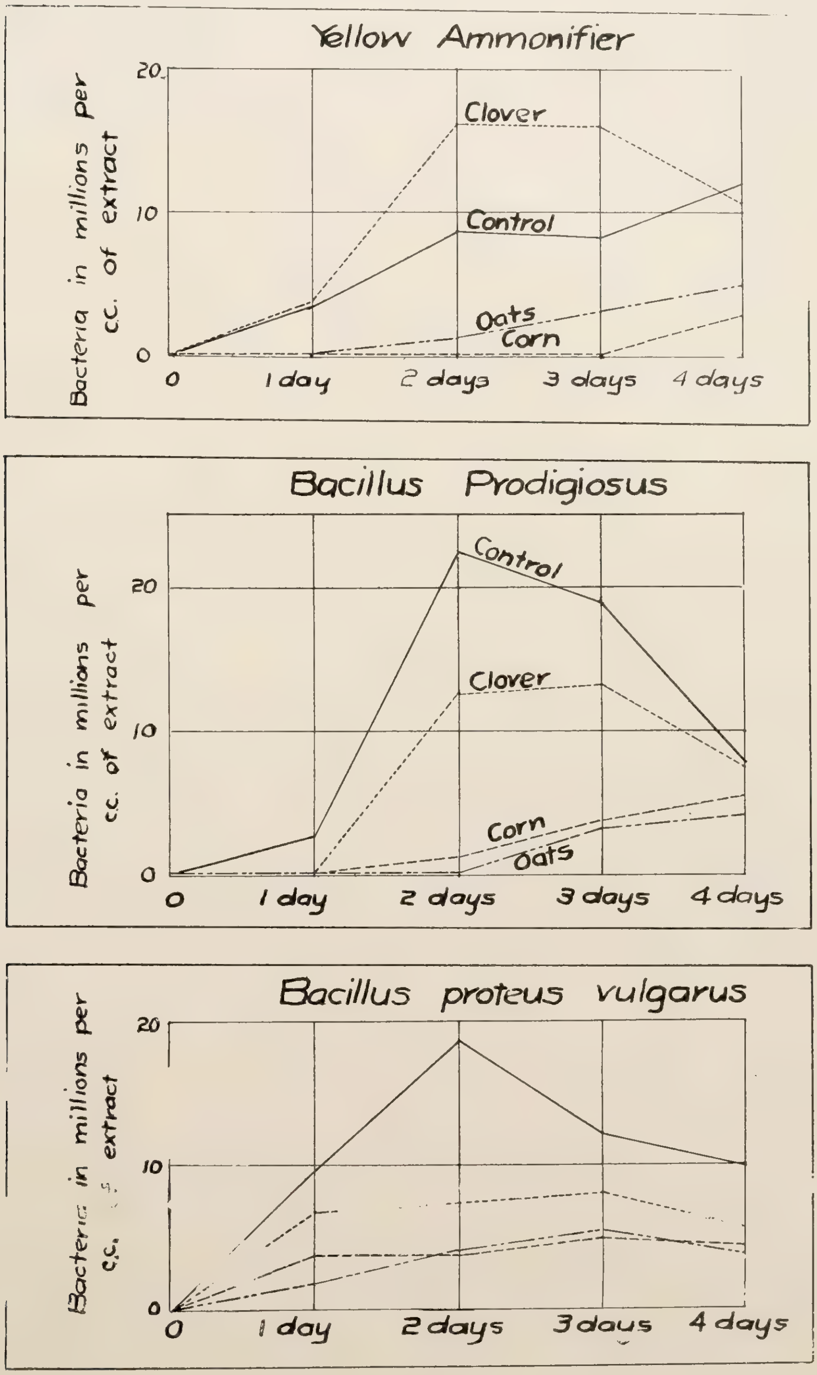


PLATE XXVIII.

FIG. 1.—*Side view of brain.* × 25.

A.—Olfactory lobes.
B.—Cerebral lobes.
C.—Optic thalami.
D.—Optic lobes.
E.—Cerebellum.
F.—Medulla.
G.—Spinal cord.
H.—Pineal body.
I.—Fossa rhomboidalis.
Par. Eye.—Parietal eye.
Inf.—Infundibulum.
Hyp.—Hypophysis.
Cil. Gang.—Ciliary ganglion.
Gass. Gang.—Gasserian ganglion.
Gen. Gang.—Geniculate ganglion.
1.—Olfactory nerve.
2.—Optic nerve.
3.—Ocular-motor nerve.
3c.—Connection with cilliary ganglion.
4.—Pathetic nerve.
5′, 5″, 5‴.—Trigeminal nerve with its three branches.
6.—Abduscent nerve.
7′, 7″, 7‴.—Facial nerve with its three branches.
8′, 8″.—Auditory nerve with its two branches.
9.—Glosso-pharyngial nerve.
10.—Vagus nerve.
11.—Spinal accessory nerve.
12.—Hypoglossary nerve.
13.—Spinal nerves.
An.—Anastomosis of the third and fifth nerves.

FIG. 2.—*Dorsal view of brain.* × 25.

All labels as in fig. 1.

FIG. 3.—*Posterior view of brain.* × 25.

All labels as in fig. 1.

PLATE XXVIII.

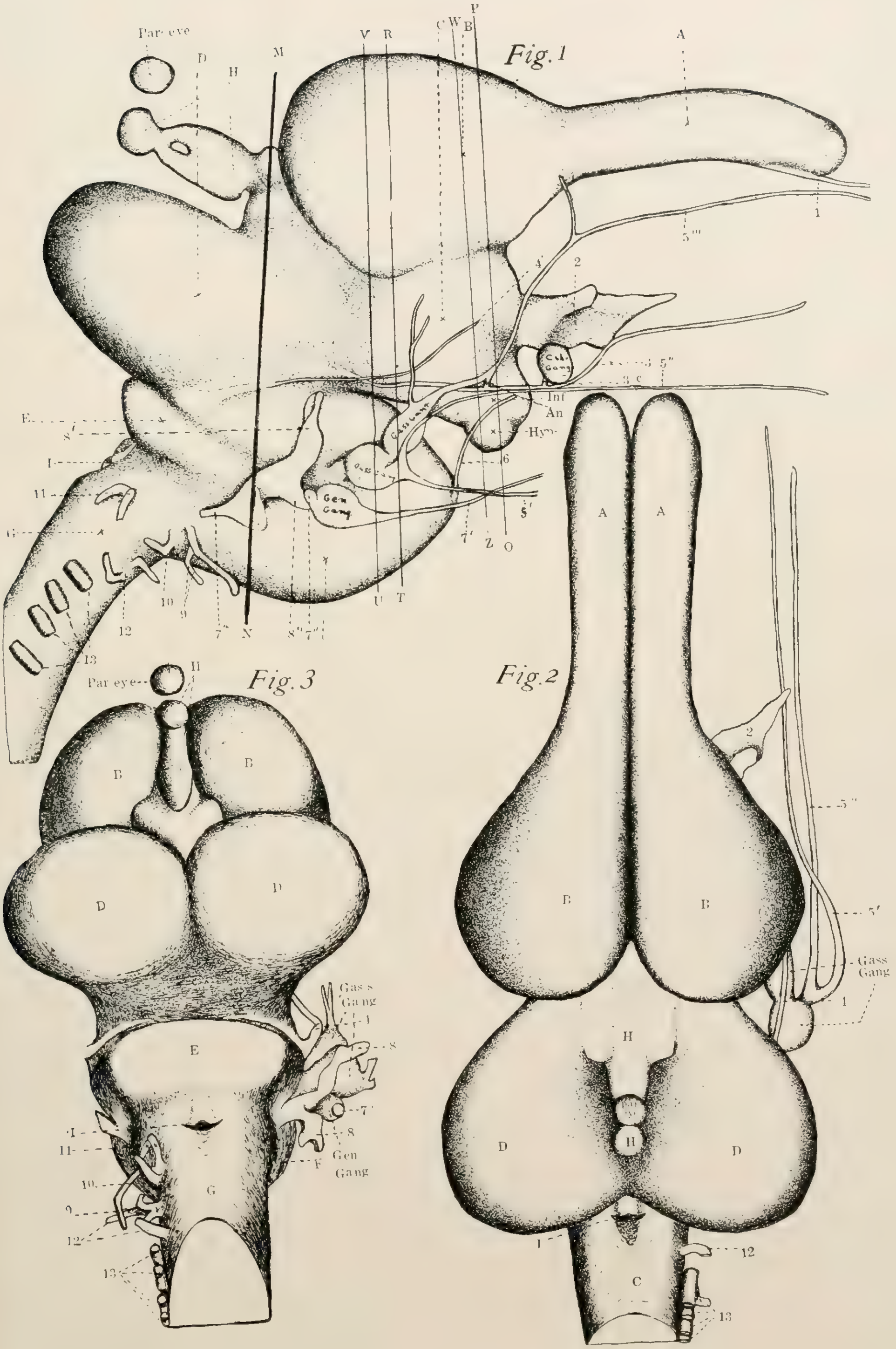


Fig. 1
Par eye
D
H
M
V
R
C
W
B
P
A
1
5'''
4'
2
Cil. Gang
3
5''
3 c
Inf
An
Hyp
Gass Gang
Gass Gang
Gen Gang
6
5'
7'
Z
O
E
8'
I
11
G
10
9
12
7''
N
8''
7'''
U
T
13
Fig. 2
A
A
2
5''
B
B
5'
Gass Gang
1
H
H
D
D
I
12
C
13
Fig. 3
Par eye
H
B
B
D
D
Gass Gang
4
8
E
7
8
4
11
10
9
12
13
G
F
Gen Gang

PLATE XXIX.

FIG. 4.—*Ventral view of brain.* × 26.

All labels as in fig. 1.

FIG. 5.—*Posterior view of model divided at MN.* × 26.

Aqu. Syl.—Aqueduct of Sylvius.
P.—Plexus.
Po.—Cavity of pineal body.
All other labels as in fig. 1.

FIG. 6.—*Anterior view of section PWZO.* × 26.

3V.—Third ventral of brain.
Ho.—Cavity of hypophysis.

PLATE XXIX.

Fig. 4

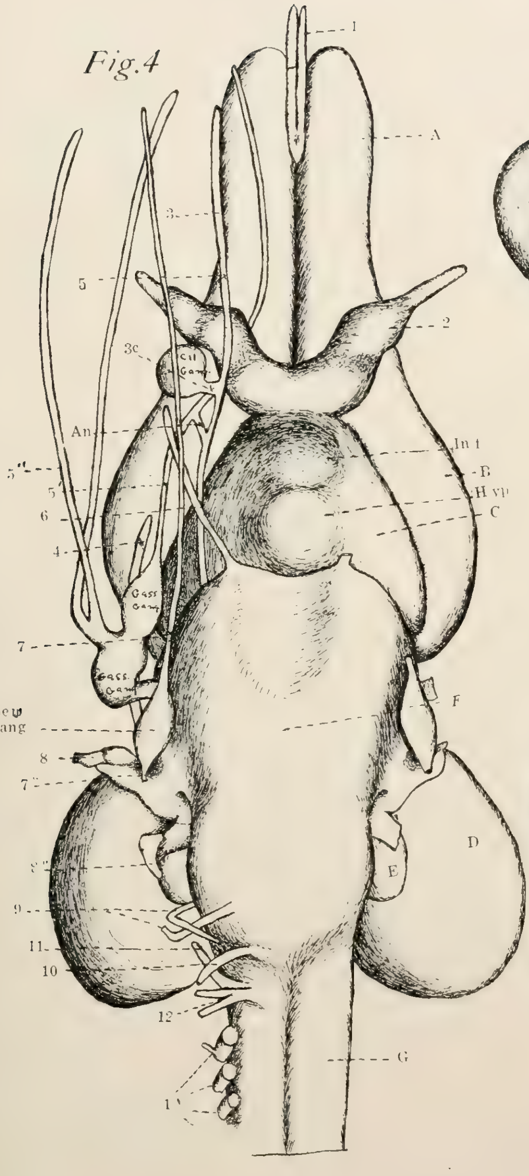
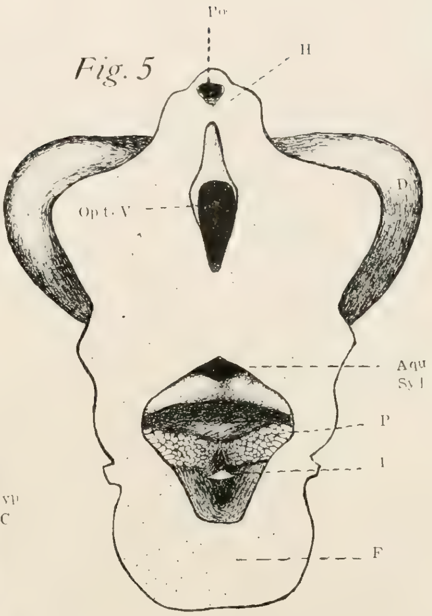

1
A
3
5
2
3c
Cil Gang
An
Int
5''
B
5'
H yp
6
C
4
Gass Gang
7
Gass Gang
F
Gen Gang
8
7''
D
E
8''
9
11
10
12
G
1'

Fig. 5


Po
H
D
Opt. V
Aqu Syl
P
1
F

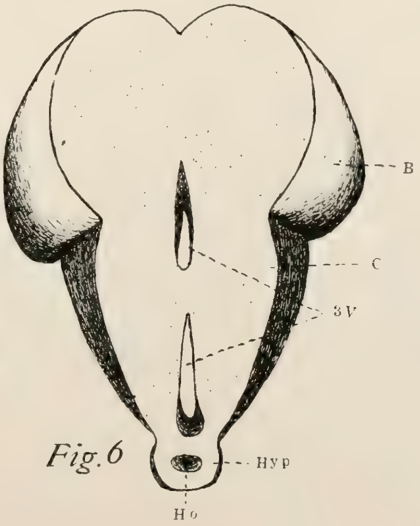


Fig. 6

PLATE XXX.

Fig. 7.—*Anterior view of section RVUT.* × 27.

3V.—Third ventricle of brain.
1V.—First ventricle of brain.
2V.—Second ventricle of brain.
For. Mon.—Foramen of Monro.
Pl.—Plexus.
All other labels as in fig. 1.

Fig. 8.—*Dorsal view illustrating brain cavities.* × 27.

Olf. V.—Olfactory ventricle.
1V.—First ventricle of brain.
2V.—Second ventricle of brain.
3V.—Third ventricle of brain.
Opt. V.—Optical ventricles.
For. Mon.—Foramen of Monro.
Sp. Cnl.—Spinal canal.

Fig. 9.—*Side view illustrating brain cavities.* × 27.

4V.—Fourth ventricle of brain.
Pl—Plexus.
Ho.—Cavity of hypophysis.
Aqu. Syl.—Aqueduct of Sylvius.
Other labels as in fig. 8.

Fig. 10.—*Diagram of cavities of pineal body and pariental eye in vertical plane.* × 54.

Fig. 11.—*Diagram of cavities of pineal body and parietal eye in horizontal plane.* × 67.

PLATE XXX.

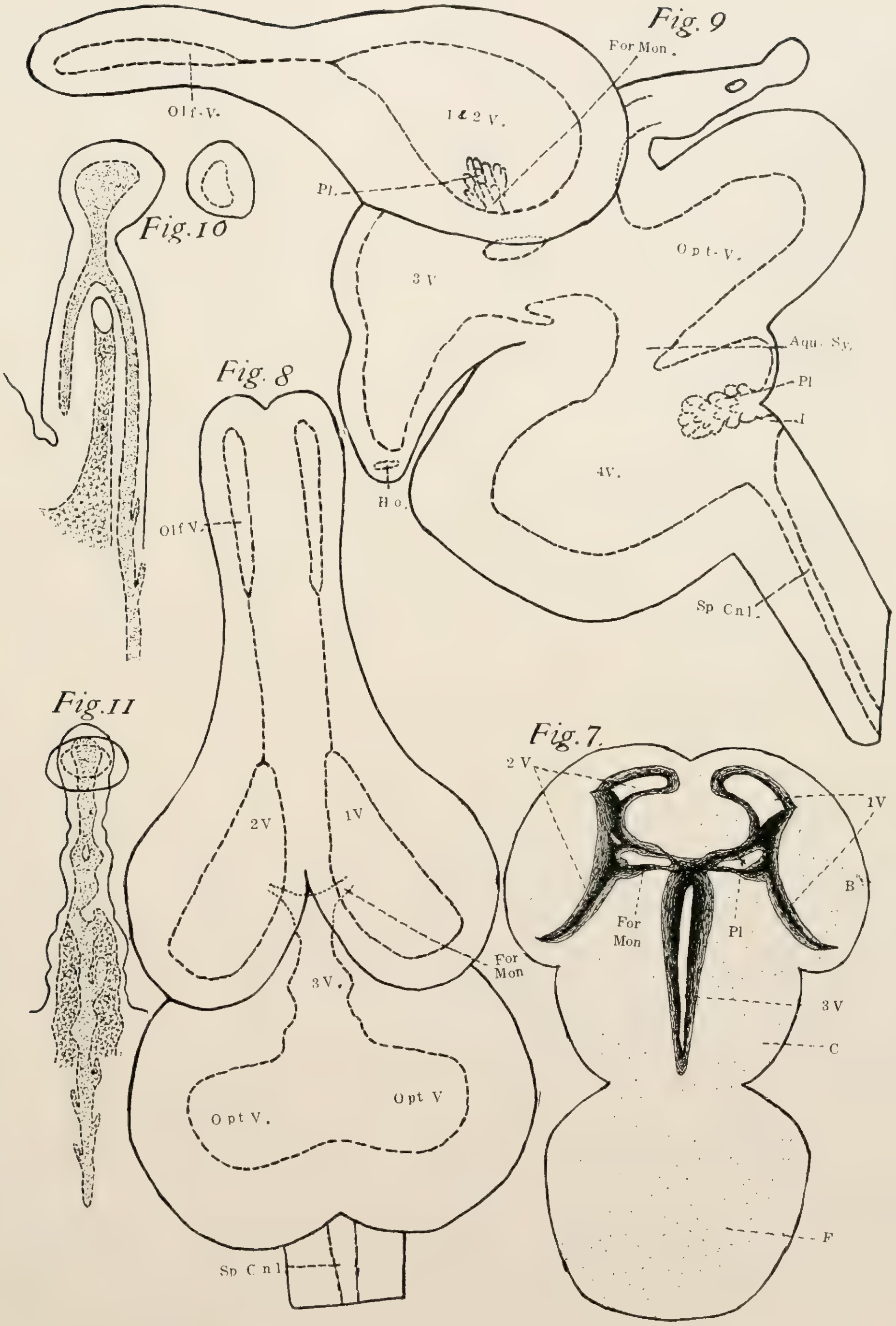


Fig. 9
For Mon.
Olf. V.
1 & 2 V.
Pl.
3 V
Opt. V.
Aqu. Sy.
Pl
I
4 V.
Ho.
Sp Cnl.
Fig. 10
Fig. 8
Olf V.
2 V
1 V
For Mon
3 V.
Opt V.
Opt V
Sp Cnl
Fig. 11
Fig. 7
2 V
1 V
B
For Mon
Pl
3 V
C
F

PLATE XXXI.

Explanation as in fig. 10

FIG. 12.—*Drawing of series of sections through optic chiasma.*

FIG. 13.—*Diagram to illustrate mode of crossing of nerves.*

FIG. 14.—*Outline drawing of adult brain.*

FIG. 15.—*Outline drawing of embryonic brain.*

PLATE XXXI.

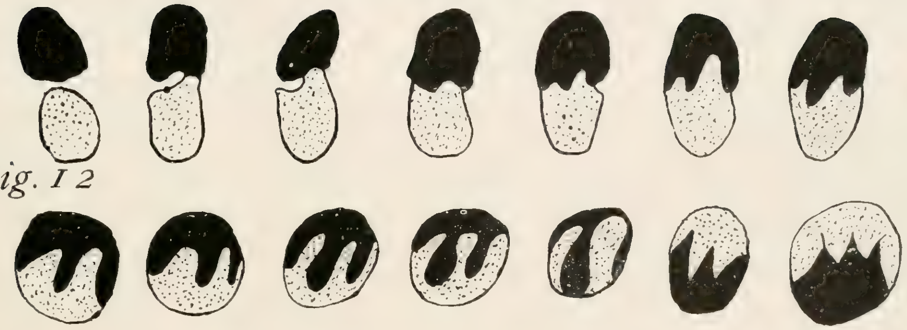


Fig. 12

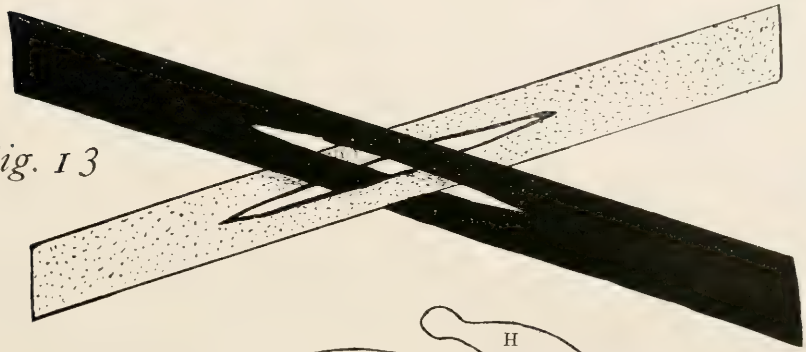


Fig. 13

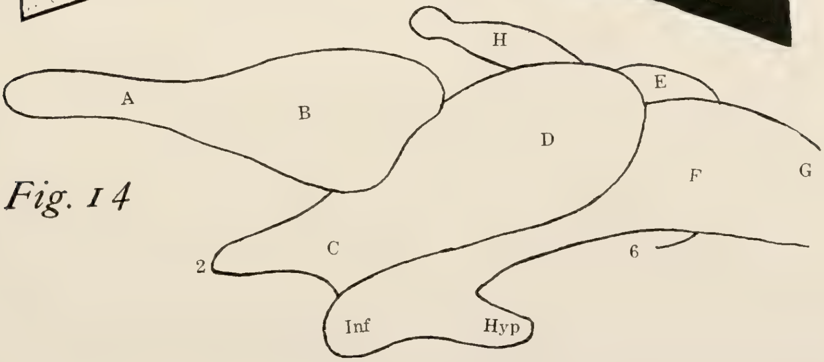


Fig. 14

H
E
A
B
D
F
G
C
2
6
Inf
Hyp

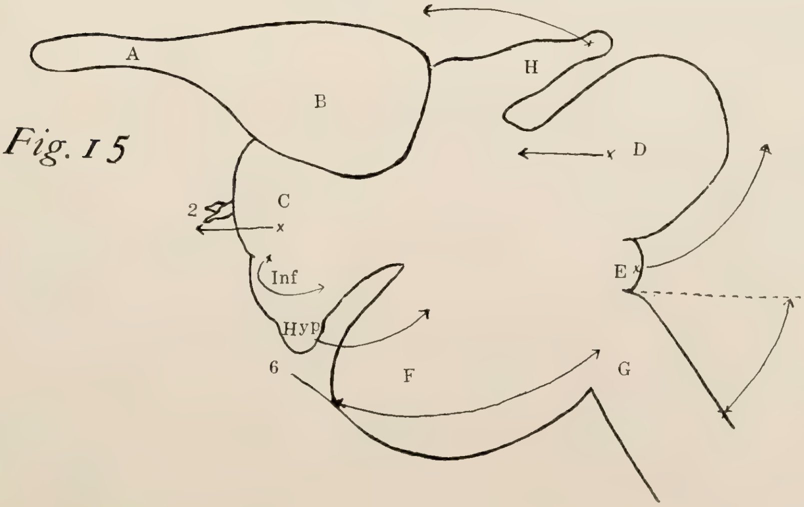


Fig. 15

A
H
B
D
C
2
Inf
E
Hyp
6
F
G

PLATE XXXII.

1. Neuroplasm. Metaphase gives an idea of the size and shape of chromosomes.
2. Ectoblast. Anaphase showing loop form as they come to the poles.
3. Ectoblast. Splitting.
4. Ectoblast. Metaphase.
5. Pigment showing full number of chromosomes and the difficulty in measuring.
6. Gryllus domesticus. First spermatocyte.
7. Muscle. Metaphase.

PLATE XXXII.

Fig. 1

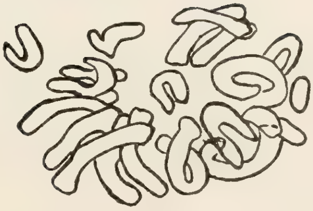


Fig. 2

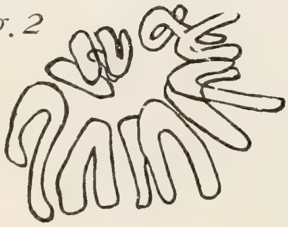


Fig. 4

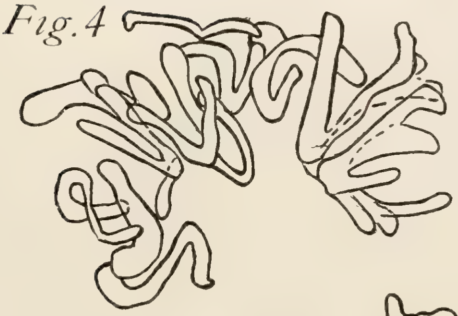
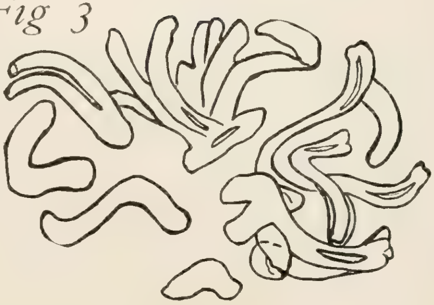


Fig 3



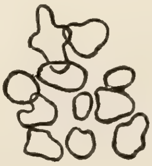


Fig 6

Fig 7

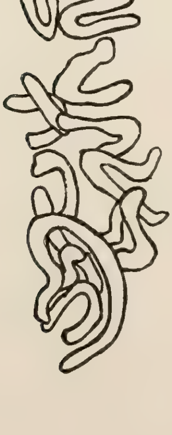


Fig 5

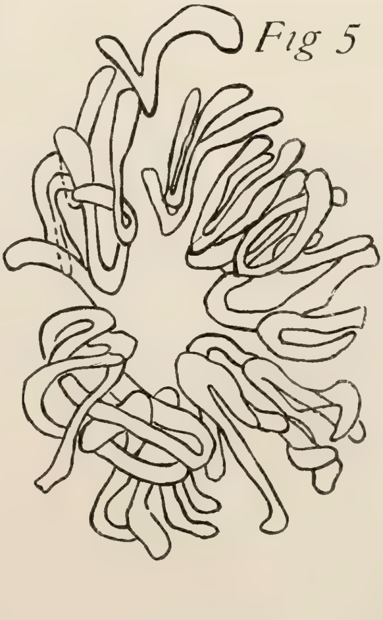


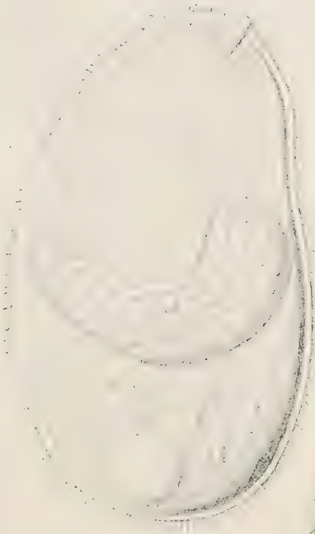
PLATE XXXIII.

Figures magnified 485 diameters.

page

FIG. 1. *Enchelydon farctus* C. & L. 152
FIG. 2. *Prorodon edentatus* C. & L. 153
FIG. 3. *Prorodon teres* Ehr. 152
FIG. 4. *Prorodon Lieberkuhnii* Bronn. 153
FIG. 5. *Coleps hirtus* Ehr. An individual that has just divided, showing absence of armature on the anterior half. 153

PLATE XXXIII.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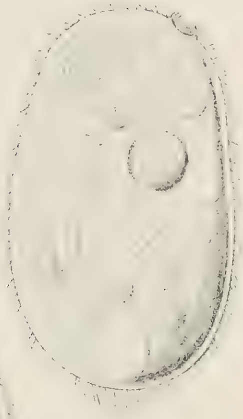
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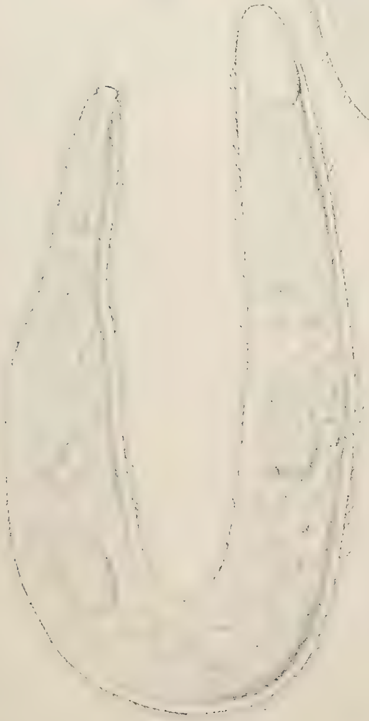
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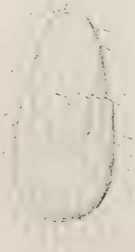
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PLATE XXXIV.

Figures magnified 485 diameters.

		page
FIG. 6.	*Lacrymaria olor* O. F. Mull	153
FIG. 7.	*Trachelius ovum* Ehr	155
FIG. 8.	*Lacrymaria olor,* contracted	153
FIG. 9.	*Coleps hirtus* Ehr	152
FIG. 10.	*Lacrymaria truncata* Stokes	153

PLATE XXXIV.

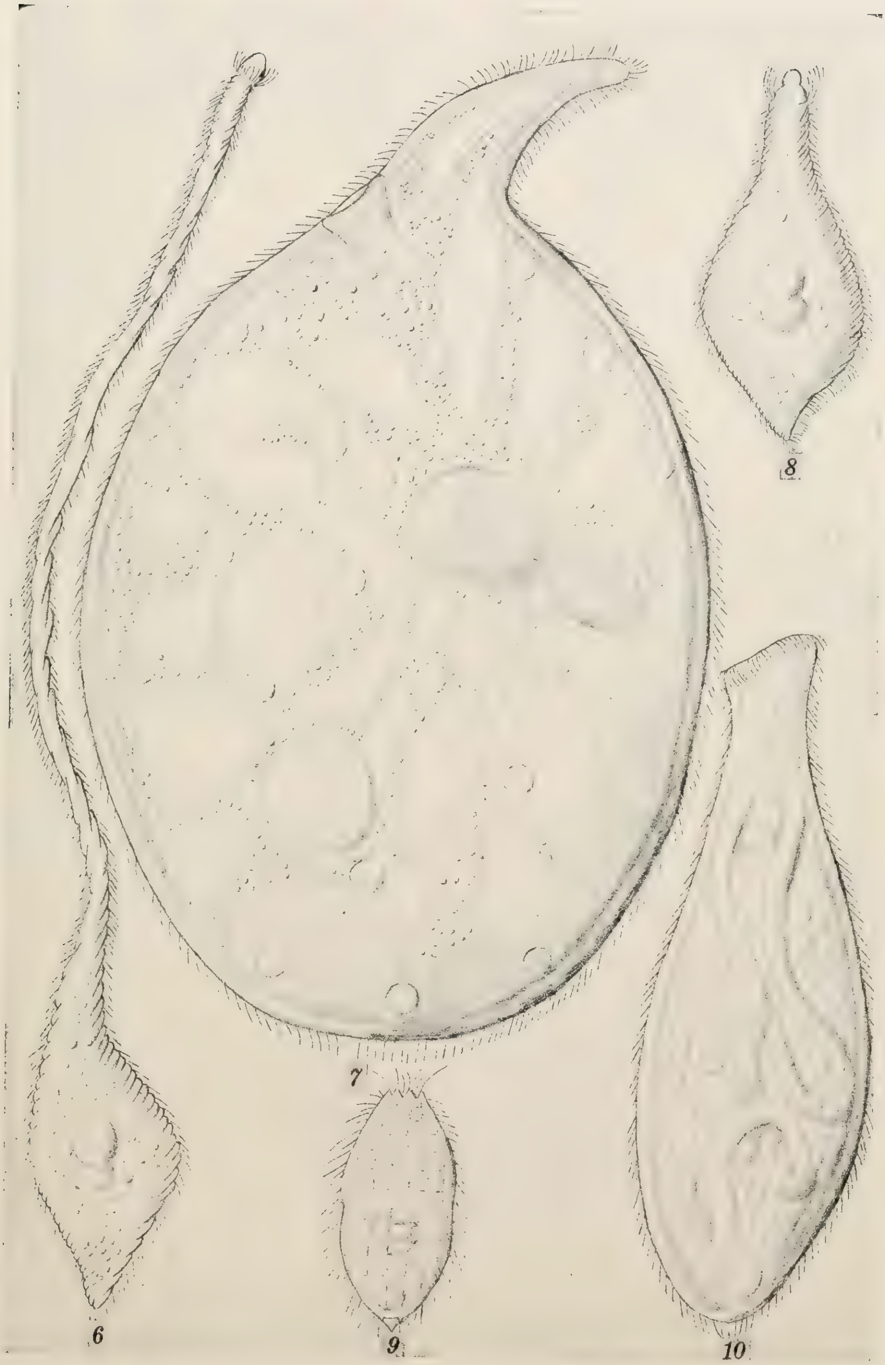
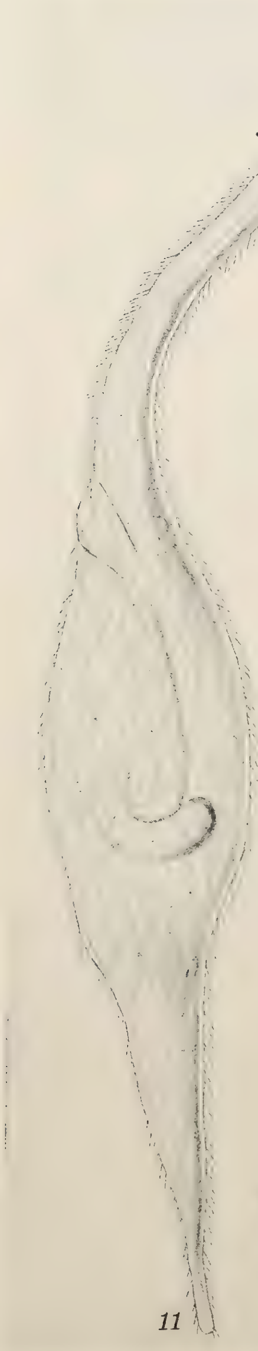


PLATE XXXV.

Figures magnified 485 diameters.

		page
Fig. 11.	*Dileptus gigas* C. & L.	154
Fig. 12.	*Loxodes rostrum* O. F. Mull	155
Fig. 13.	*Chilodon cucullus* Mull	156
Fig. 14.	*Lionotus Urzesniowskii* S.K	154
Fig. 15.	*Lionotus fasciola* Ehr	154
Fig. 16.	*Chilodon megalotrocha* Stokes	157

PLATE XXXV.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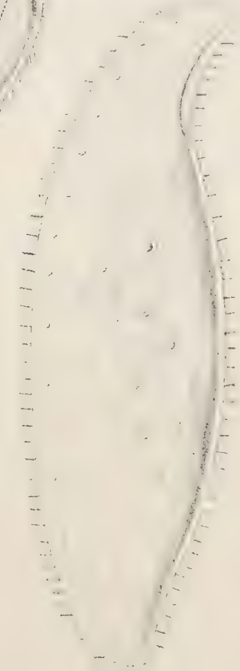
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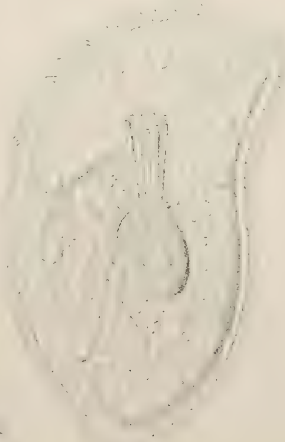
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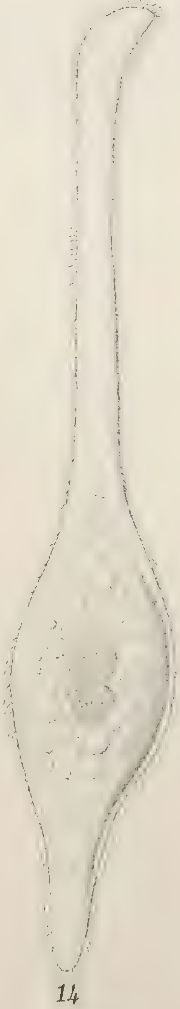
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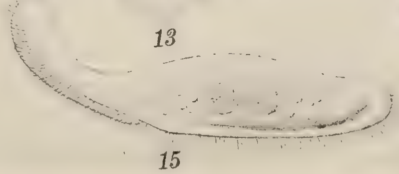
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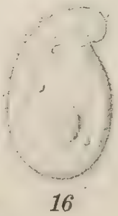
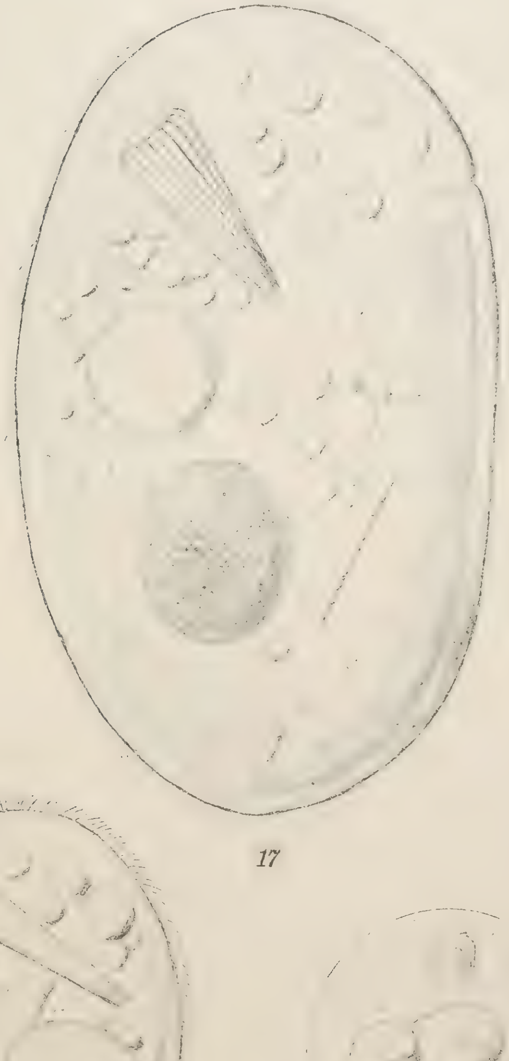

16

PLATE XXXVI.

Figures magnified 485 diameters.

		page
FIG. 17.	*Nassula aurea* Ehr	156
FIG. 18.	*Nassula rubens* C. & L	156
FIG. 19.	*Nassula rubens* encysted	156

PLATE XXXVI.



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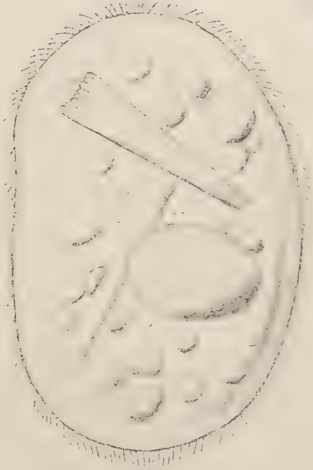

18

19

PLATE XXXVII.

Figures magnified 485 diameters.

		page
FIG. 20.	*Colpoda cucullus* Ehr	158
FIG. 21.	*Colpoda helia* Stokes	158
FIG. 22.	*Frontonia leucas* Ehr	157
FIG. 23.	*Colpidium putrinum* Stokes	158
FIG. 24.	*Colpidium striatum* Stokes	158

PLATE XXXVII.

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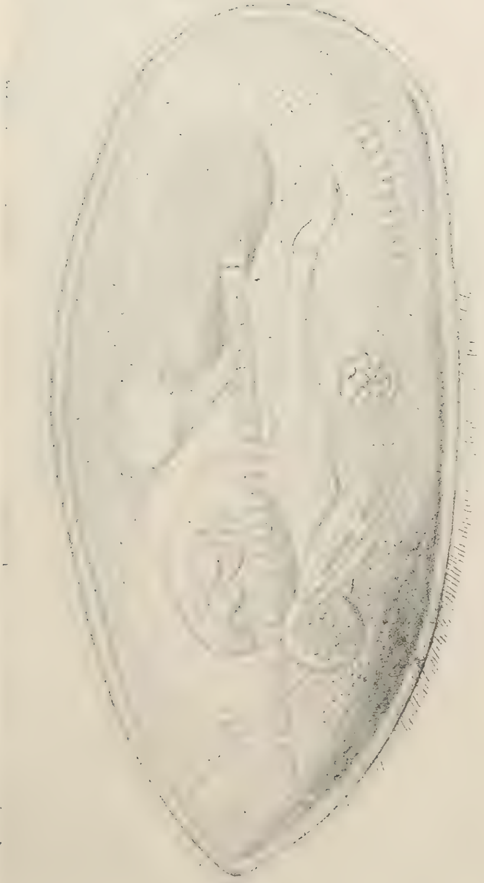

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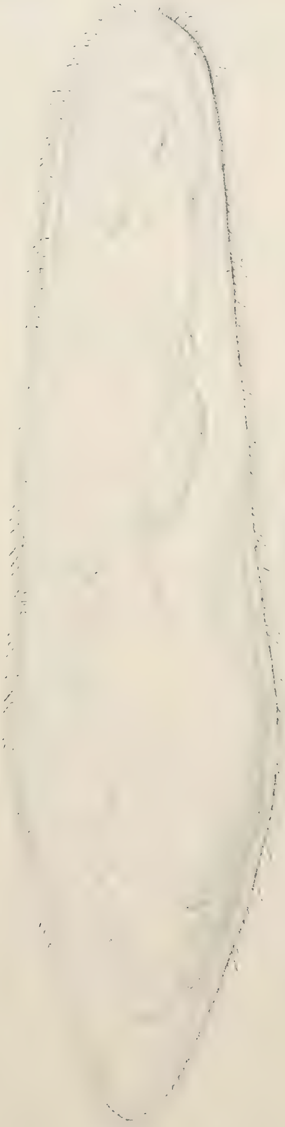
PLATE XXXVIII.

Figures magnified 485 diameters.

		page
FIG. 25.	*Paramæcium caudatum* Ehr.	159
FIG. 26.	*Tillina magna* Gruber	159
FIG. 27.	*Cyclidium glaucoma* Ehr	160
FIG. 28.	*Paramæcium bursaria* Ehr.	159

PLATE XXXVIII.



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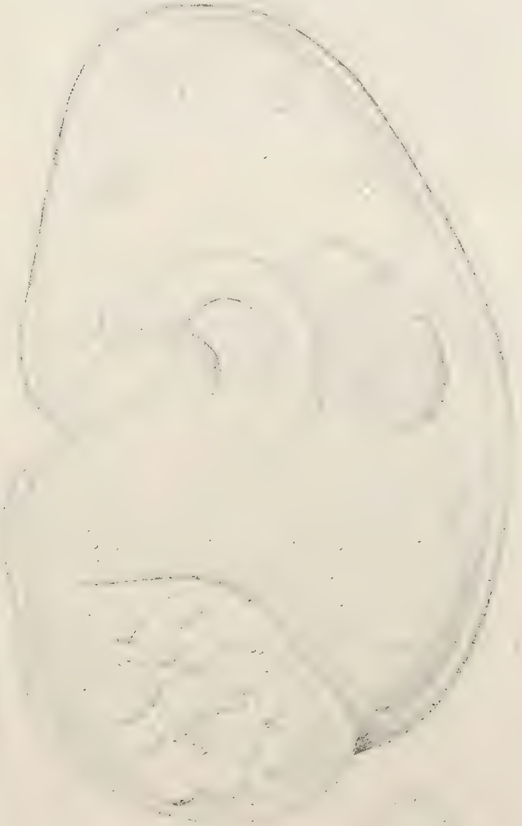

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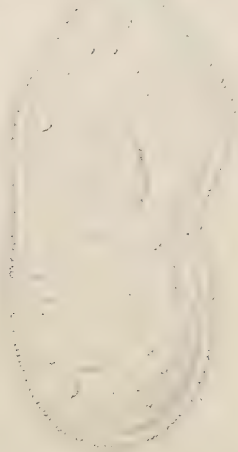

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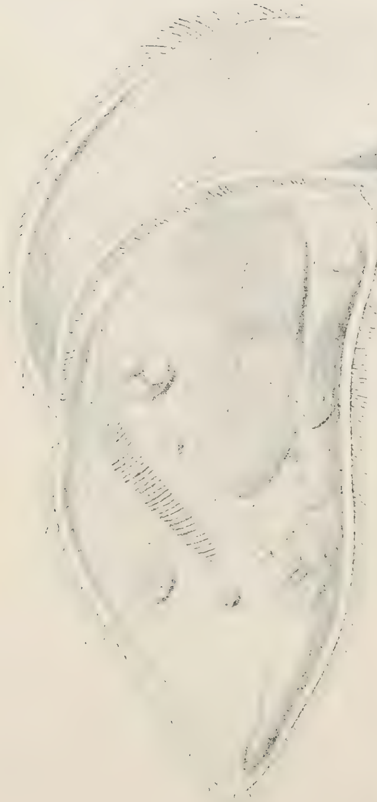
PLATE XXXIX.

Figures magnified 485 diameters.

		page
FIG. 29.	*Metopus sigmoides* Mull	161
FIG. 30.	*Condylostoma stagnale* Wrz	162
FIG. 31.	*Metopides acuminata* Stokes	161
FIG. 32.	*Nyctotherus cordiformis* Stein	160

PLATE XXXIX.



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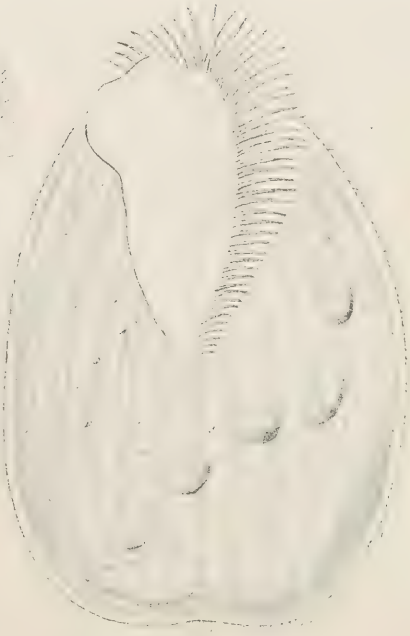

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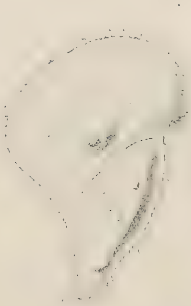

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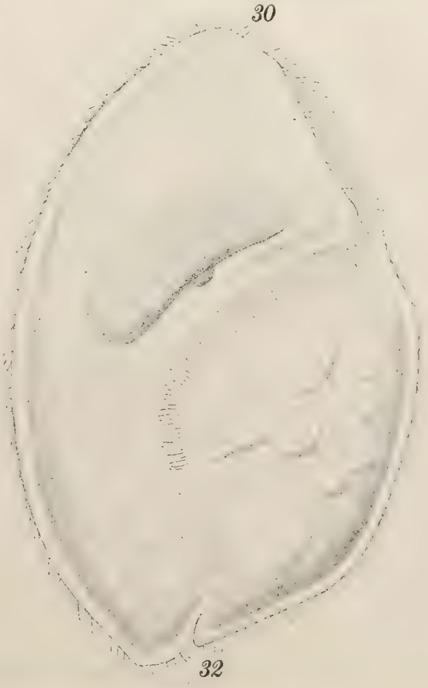

32

PLATE XL.

Figures 33-35 magnified 200 diameters; figure 36 magnified 485 diameters.

		page
Fig. 33.	*Spirostomum teres* C. & L.	162
Fig. 34.	*Stentor roeselii* Ehr.	163
Fig. 35.	*Spirostomum ambiguum* Ehr.	161
Fig. 36.	*Halteria grandinella* Mull.	163

PLATE XL.

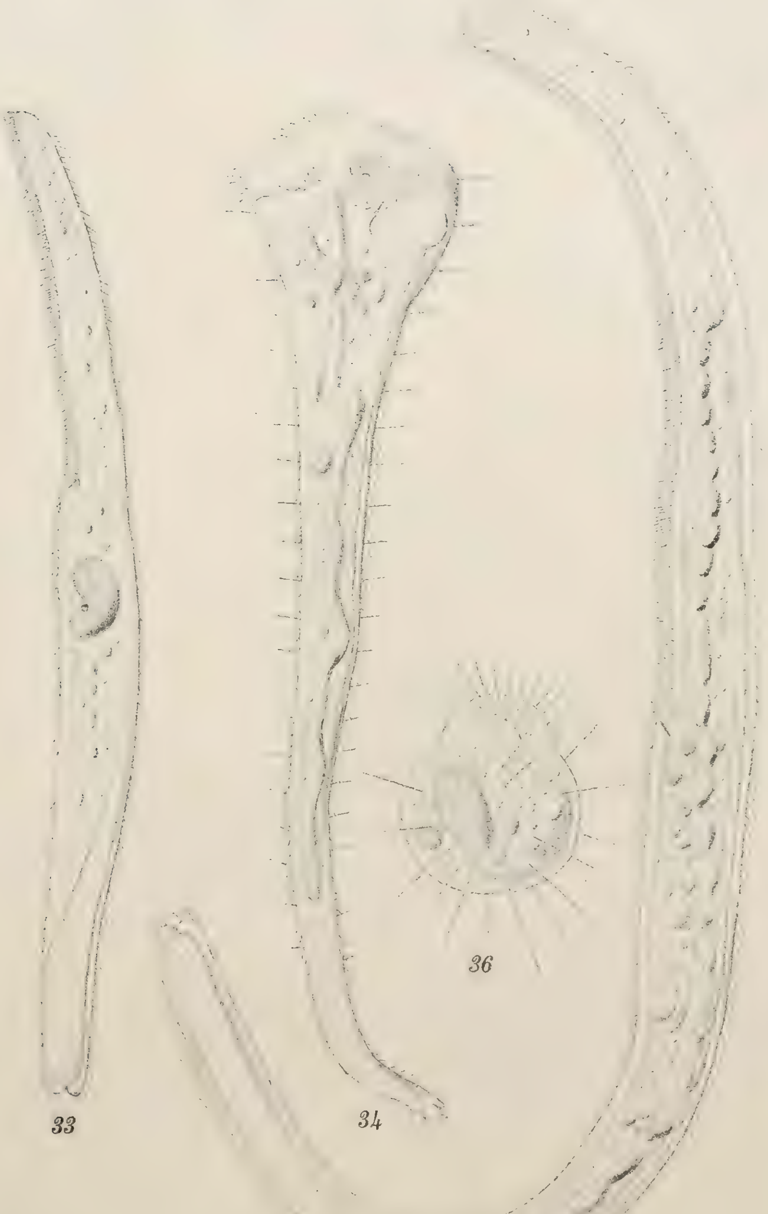

PLATE XLI.

Figure 37 magnified 200 diameters.

		page
FIG. 37.	*Stentor caeruleus* Ehr.	163
FIG. 38.	*Stentor cæruleus.* Low-power drawing of completely expanded animal	163

PLATE XLI.

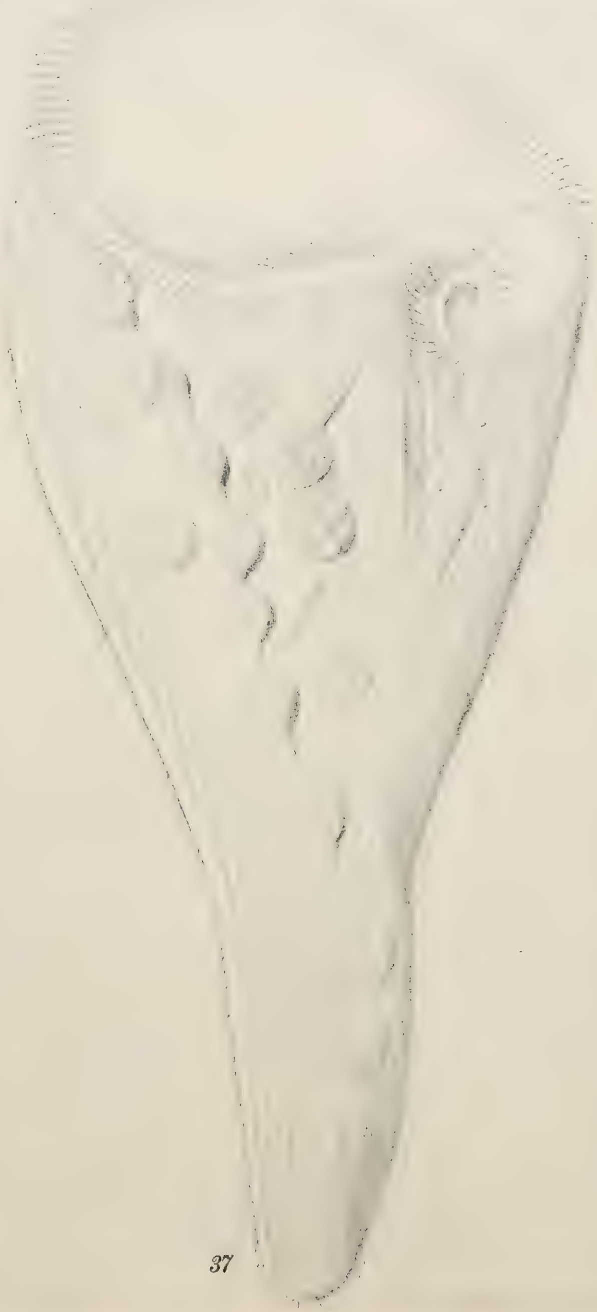

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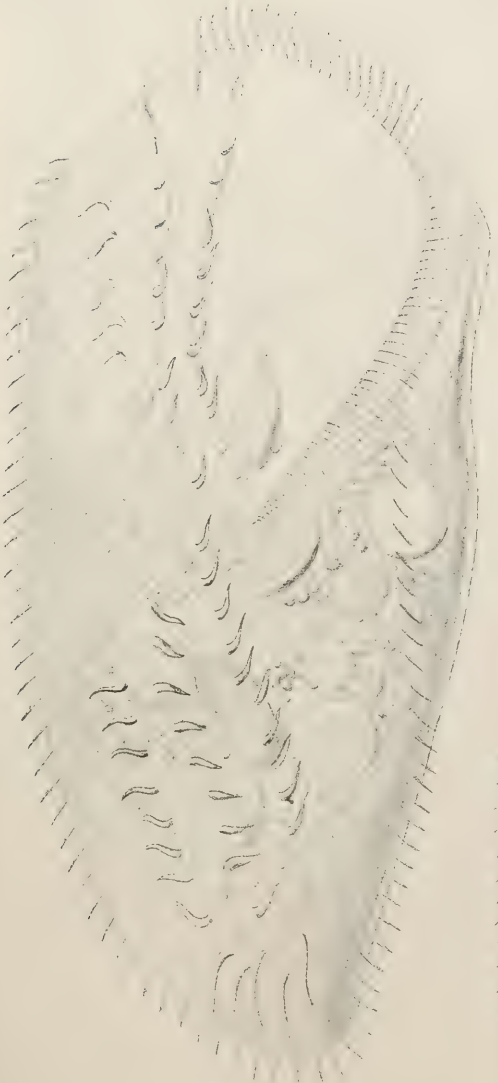
PLATE XLII.

Figures magnified 485 diameters.

		page
FIG. 39.	*Onychodromus grandis* Stein	165
FIG. 40.	*Urpleptus agilis* Eng	164
FIG. 41.	*Gastrostyla Steinii* Eng	165

PLATE XLII.



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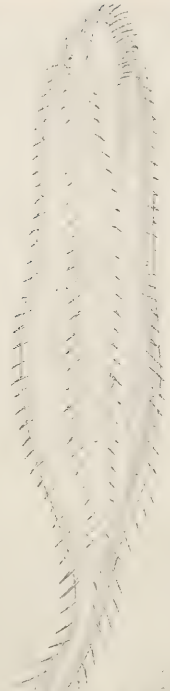

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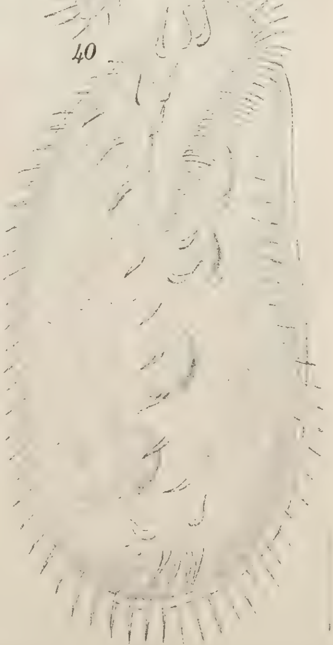

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PLATE XLIII.

Figures magnified 485 diameters.

		page
FIG. 42.	*Stylonychia pustulata* Ehr	166
FIG. 43.	*Stylonychia mytilus* Ehr	166
FIG. 44.	*Stylonychia notophora* Stokes	166
FIG. 45.	*Oxytricha bifaria* Stokes	165
FIG. 46.	*Oxytricha pellionella* Mull	166

PLATE XLIII.

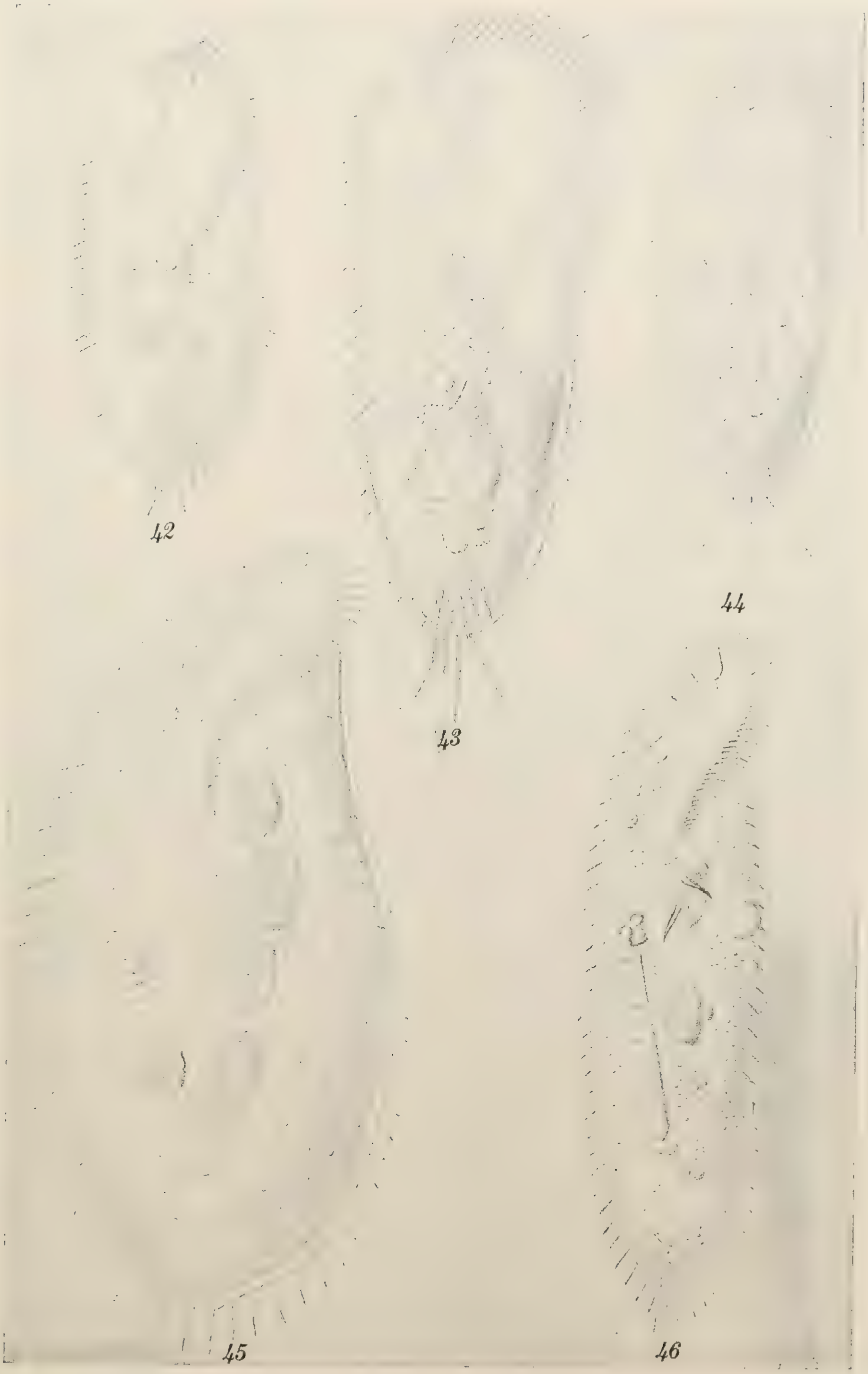

42
43
44
45
46

PLATE XLIV.

Figures magnified 485 diameters.

page

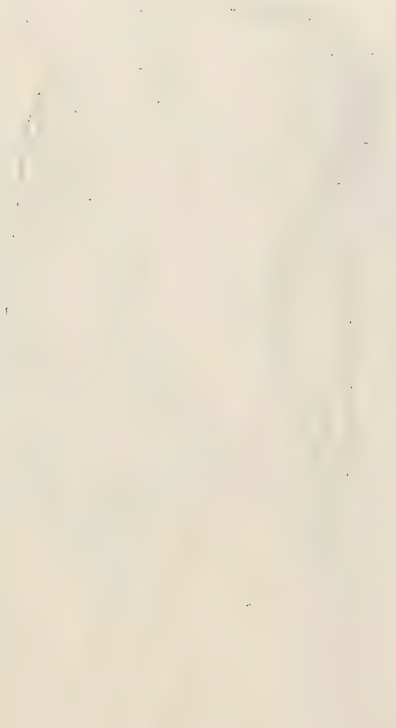
FIG. 47. *Euplotes patella* Ehr. 167

FIG. 48. *Urostyla grandis* Ehr. 167

FIG. 49. *Euplotes carinata* Stokes. 167

FIG. 50. *Aspidisca costata* Duj. 167

PLATE XLIV.



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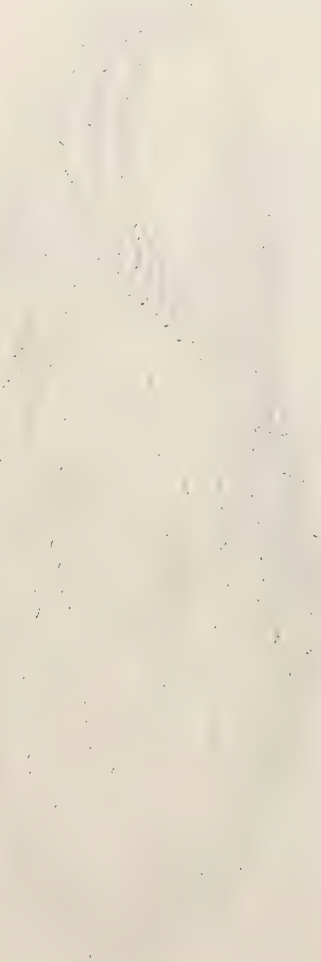

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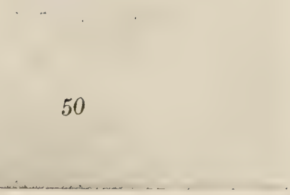

50

PLATE XLV.

Figures magnified 485 diameters.

		page
FIG. 51.	*Vorticella cucullus* From	170
FIG. 52.	*Vorticella longifilum* S. K	170
FIG. 53.	*Vorticella gracilis* Duj	169
FIG. 54.	*Vorticella microstoma* Ehr	170
FIG. 55.	*Vorticella convallaria* Linn	171
FIG. 56.	*Vorticella aquae-dulcis* Stokes	171
FIG. 57.	*Vorticella simulis* Stokes	171
FIG. 58.	*Vorticella pusilla* Stokes	171
FIG. 59.	*Vorticella campanula* Ehr	170

PLATE XLV.

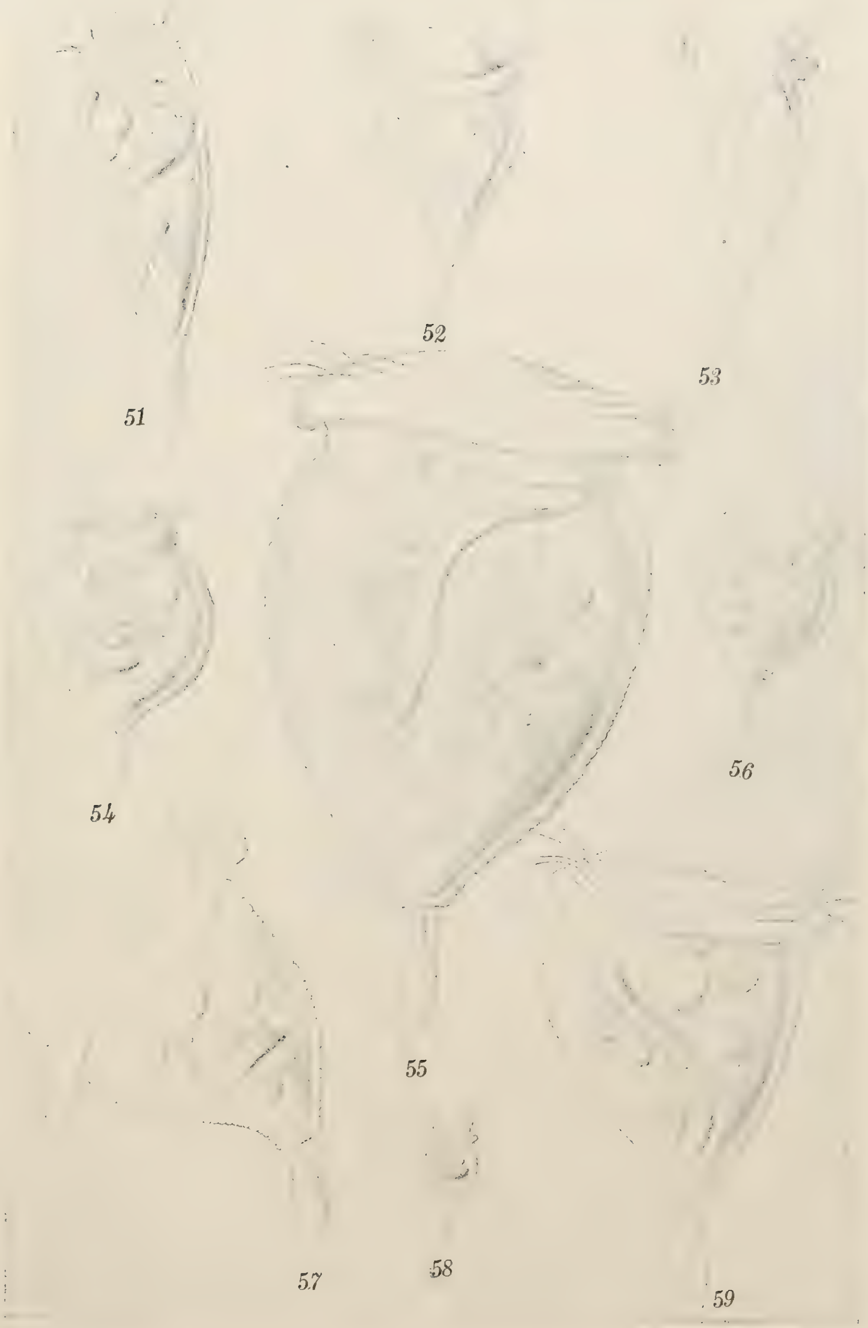
51
52
53
54
55
56
57
58
59

PLATE XLVI.

Figures magnified 485 diameters.

		page
FIG. 60.	*Vorticella elongata* From	170
FIG. 61.	*Vorticella utriculus* Stokes	170
FIG. 62.	*Vorticella spectabilis* S. K.	170
FIG. 63.	*Scyphidia inclinans* D'Udk	169
FIG. 64.	*Telotrochidium* sp	168
FIG. 65.	*Vaginicola crystallina* (?) Ehr	173
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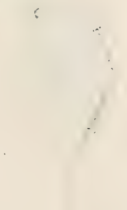
PLATE XLVI.

60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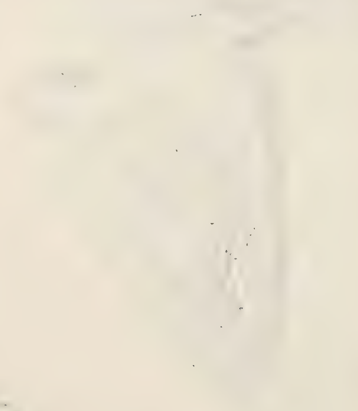
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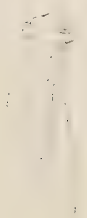
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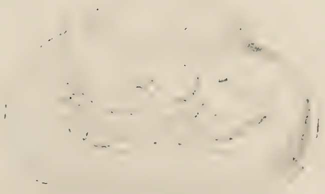
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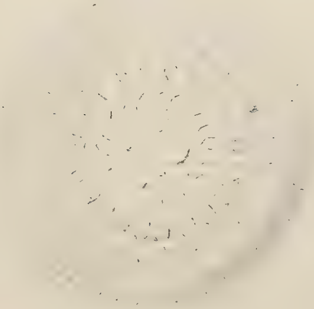
68

PLATE XLVII.

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PLATE XLVII.

69

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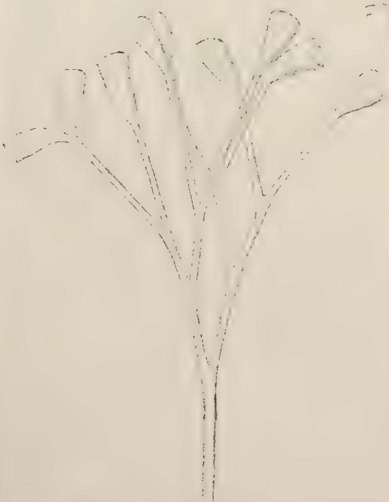

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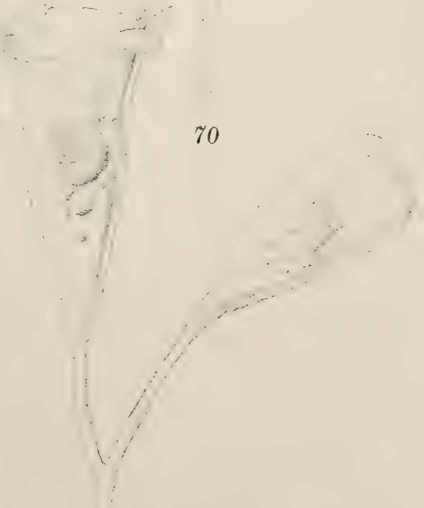

72

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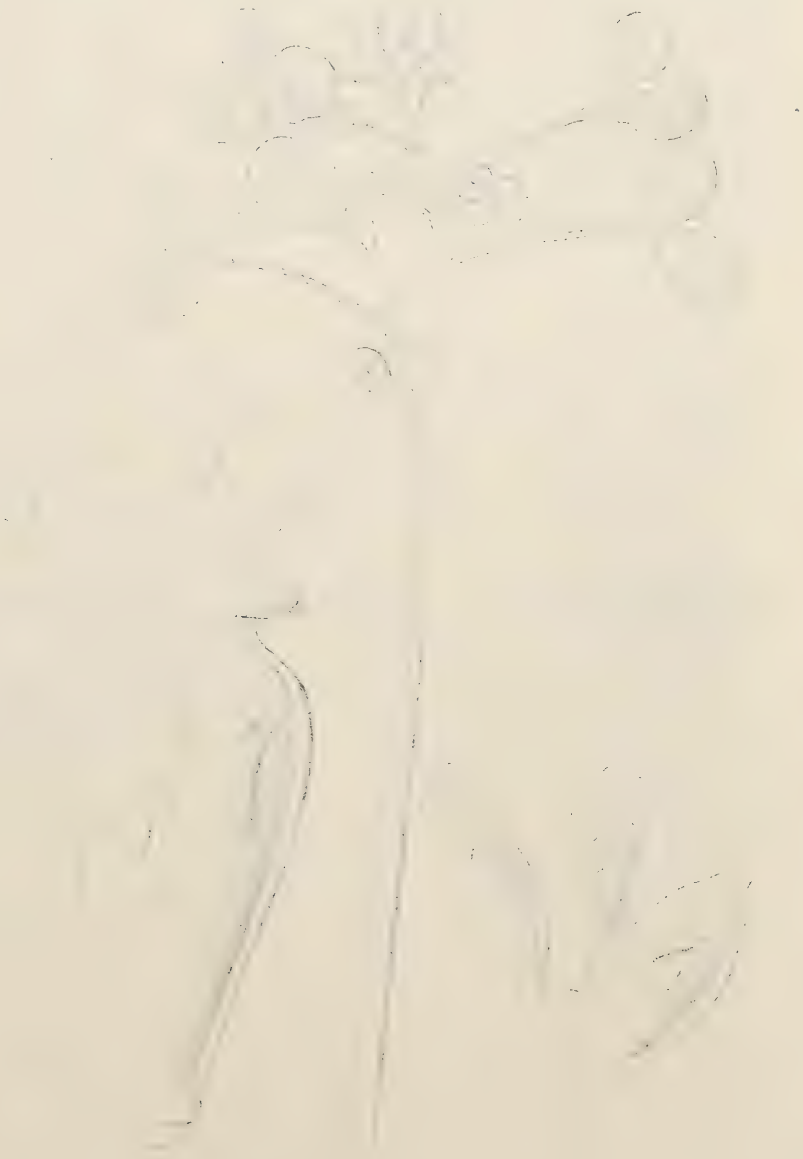

73 74 75

PLATE XLIX.

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PLATE XLIX.

76

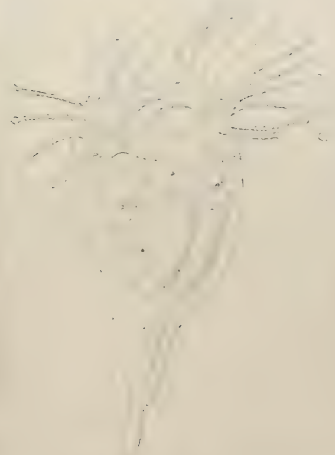

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PLATE L.

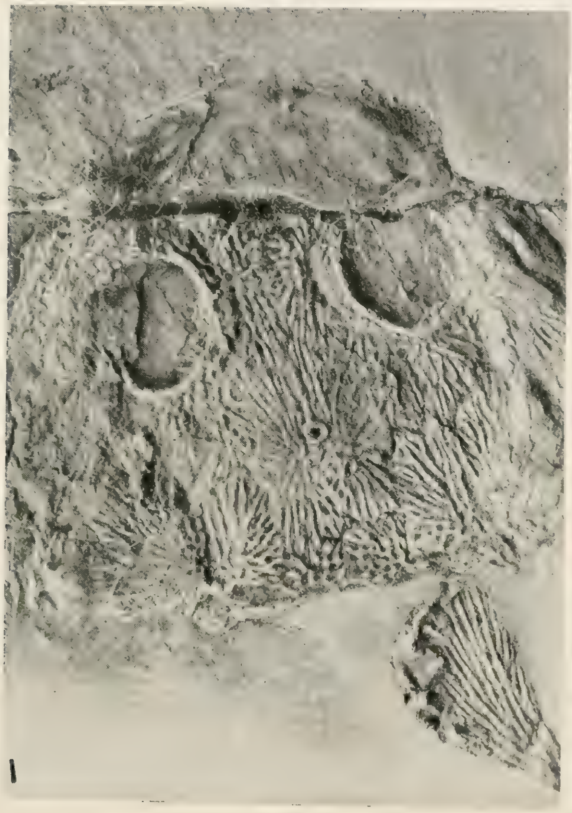
FIGURE 1. Skull of *Erpetosaurus tabulatus* Cope, showing a perfect application of the method of using the ammonium chloride deposit. The effect had been heightened by the application of a white wash to the background of matrix. It will be clearly seen that all of the details of the skull structure are evident. This method is especially useful in studying the course of the lateral line canals on the small amphibian skulls. The supraorbital canals are thus indicated as shadows above the orbits and were not previously observed.

FIGURE 2. Skull of *Erpetosaurus lævis* Moodie, showing the adaptability of the method to irregular surfaces. The membranous skull bones had become loosened from this cast and left only their impressions. The block of coal is much broken, yet there are many details which are evident on close study.

FIGURE 3. An example of an imperfect fossil, representing a form identified by Cope as *Molgophis wheatleyi* and showing an improper use of the ammonium chloride deposit. The upper part of the fossil is not covered uniformly with the rest, and will illustrate the advantage of the ammonium chloride deposit as an illuminant for dark surfaces.

FIGURE 4. The fossil is a portion of the skeleton of *Ptyonius nummifer* Cope, and illustrates the disadvantages of the deposition of too much ammonium chloride on the specimen. On removing the deposit by breathing on it and making another more tenuous deposit the fossil was clearly evident.

PLATE L.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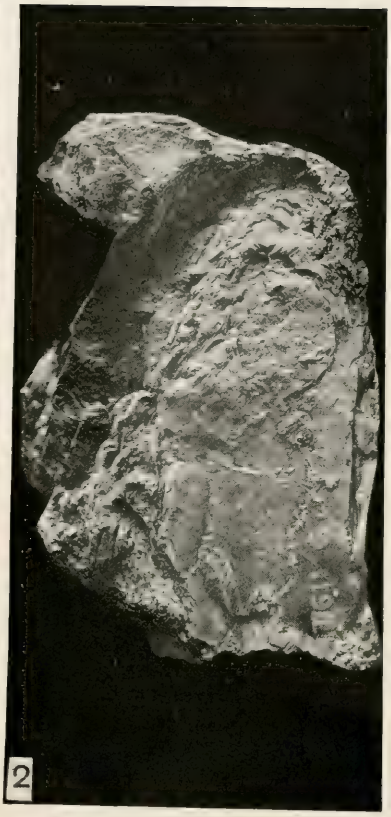
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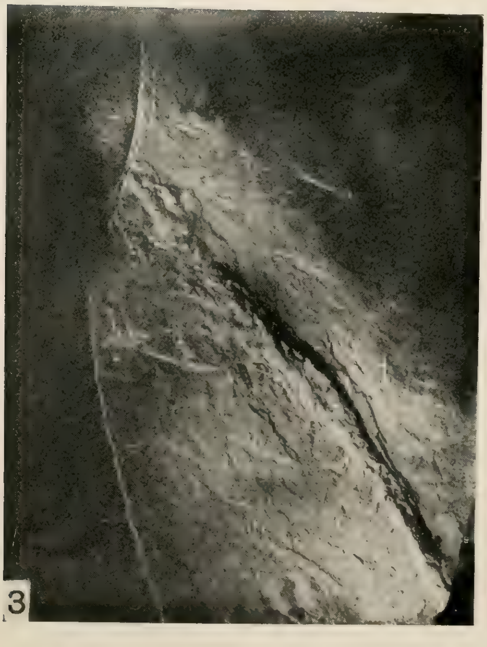
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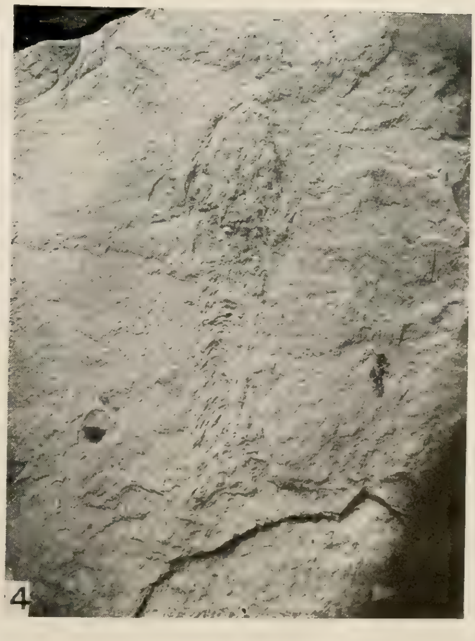
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PLATE LI.

Fig. 1.—Longitudinal section through the ectoderm, entoderm and mesoglea of the peduncle at the base of a bud, showing the germ cells in the ectoderm migrating toward the bud.

Fig. 2.—Transverse section through the peduncle in the region of a developing bud, showing germ cells in the entoderm. Stage A.

Fig. 3.—Median longitudinal section through a medusa bud, showing germ cells in the entoderm and the thickening of the ectoderm at the apex. Stage B.

ABBREVIATIONS FOR ALL FIGURES.

B. Ent., Bud entoderm.
Cav. Man., Cavity of the manubrium.
Cav. Med., Cavity of the medusa.
E., Egg.
Ect., Ectoderm.
Ect. Th., Ectodermal thickening.
Ent., Entoderm.
Ent. Man., Entoderm of the manubrium.
Ent. Umb., Entoderm of the umbrella.
Ext. Ect. Umb., External ectodermal layer of the umbrella.
Ext. Glk., External layer of the "glockenkern."
Int. Ect. Umb., Internal ectodermal layer of the umbrella.
Int. Glk., Internal layer of the "glockenkern."
G. C., Germ cell.
Mes., Mesoglea.
Nt., Nematocyst cell.
R. C., Radial canal.
S. C., Somatic cell.
Spm., Sperm.

PLATE LI.

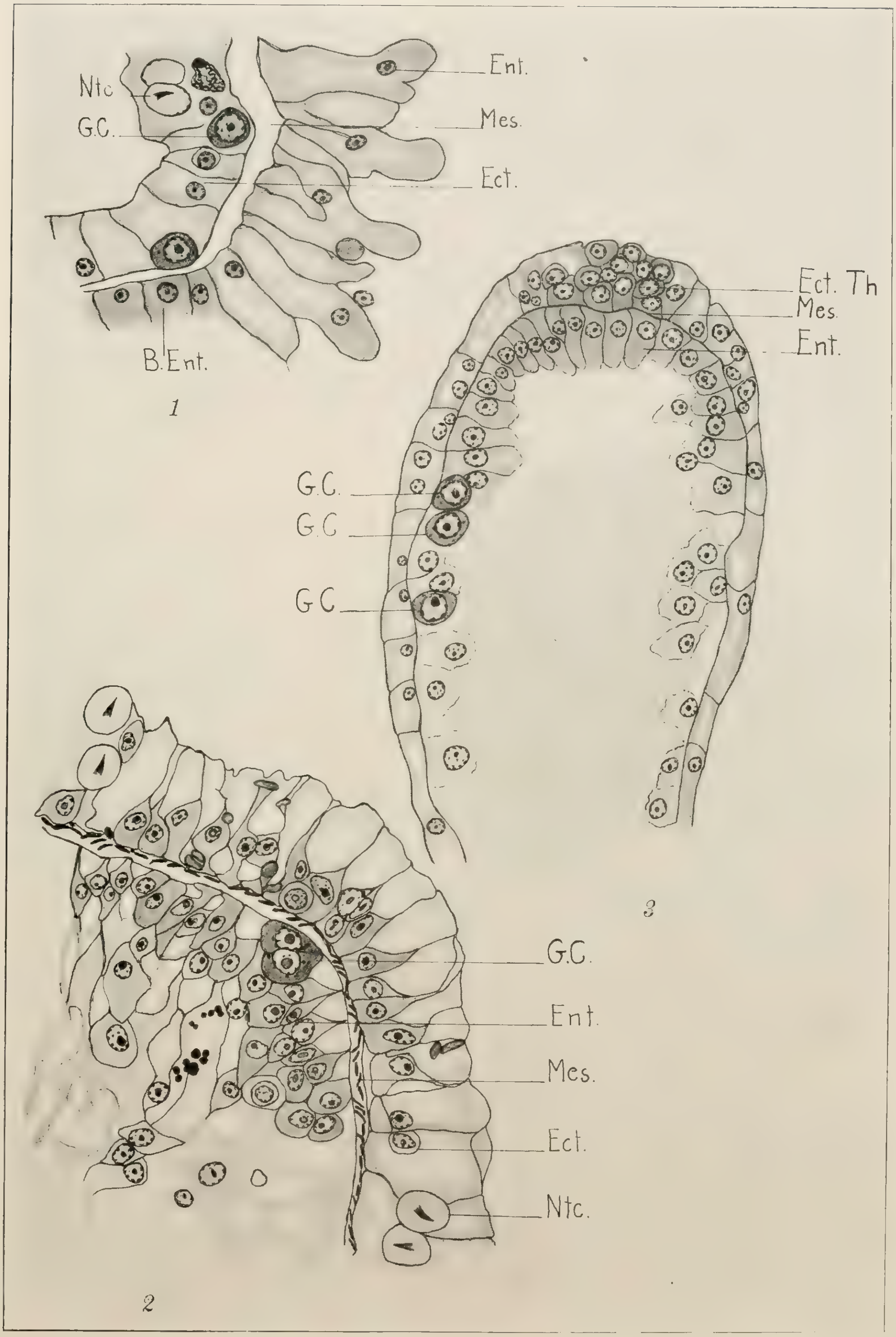

Ntc
G.C.
Ent.
Mes.
Ect.
B.Ent.
1
Ect. Th
Mes.
Ent.
G.C.
G.C
G.C
3
G.C.
Ent.
Mes.
Ect.
Ntc.
2

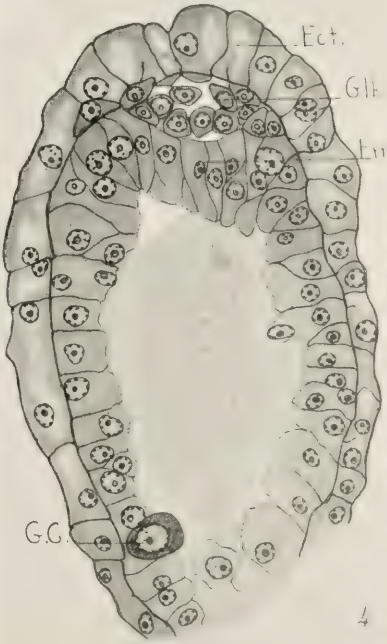
PLATE LII.

Fig. 4.—Median longitudinal section through a bud, showing the formation of the "glockenkern" at the apex. Stage C.

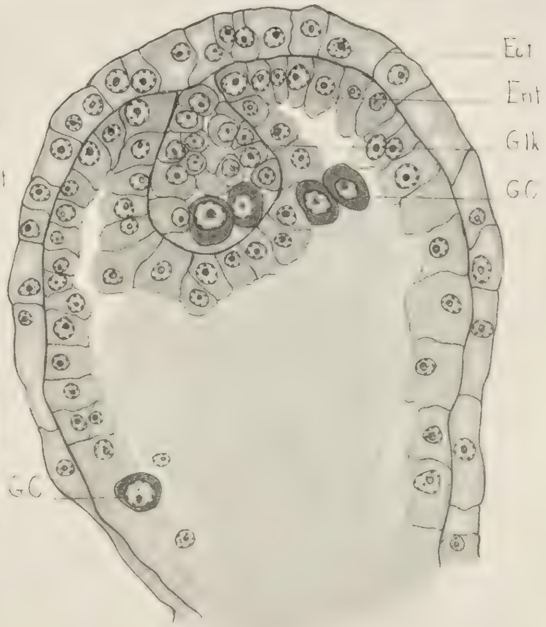
Fig. 5.—Median longitudinal section through a bud, showing germ cells migrating into the pear-shaped "glockenkern." Stage D.

Fig. 6.—Median longitudinal section through a bud, showing the separation of the two layers of the "glockenkern" cell mass and the formation of the manubrium. Stage E.

PLATE LII.


Ect.
Glk
Ent
G.C.

4


Ect
Ent
Glk
G.C
G.C

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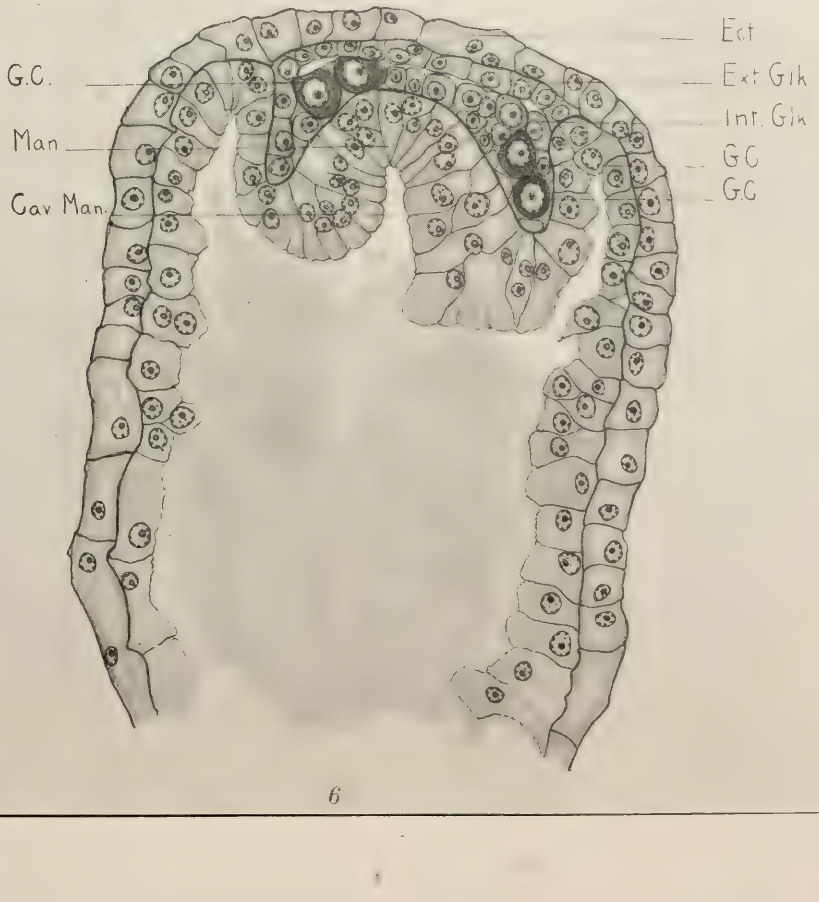

Ect
G.C.
Ext Glk
Int. Glk
Man
G.C
G.C
Cav Man.

6

PLATE LIII.

FIG. 7.—Semidiagrammatic median longitudinal section through a bud, showing complete differentiation of all the cell layers.

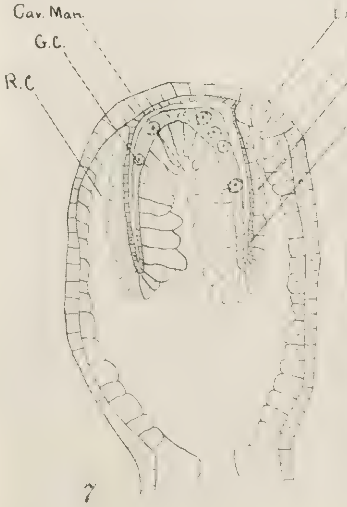
FIG. 8.—Semidiagrammatic transverse section through a medusa at the same stage of development as the one shown in figure 7, showing the cell layers and the radial canals.

FIG. 9.—Semidiagrammatic median longitudinal section through a medusa, showing the manubrium breaking through the outer capsule.

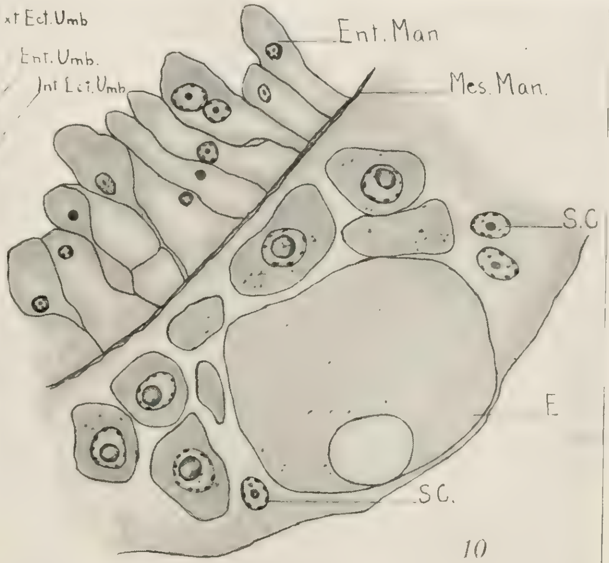
FIG. 10.—Segment of a transverse section through the manubrium of an older female medusa.

FIG. 11.—Segment of a transverse section through the manubrium of a male medusa, with a diameter equal to that of the medusa shown in figure 10.

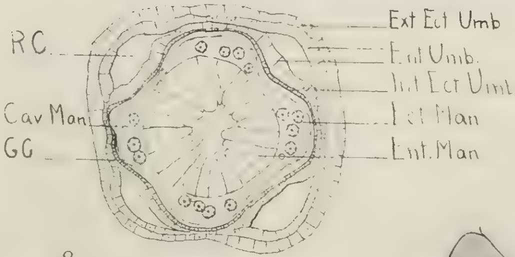
PLATE LIII.


Cav. Man.
G.C.
R.C
Ext Ect. Umb
Ent. Umb.
Int Ect. Umb.

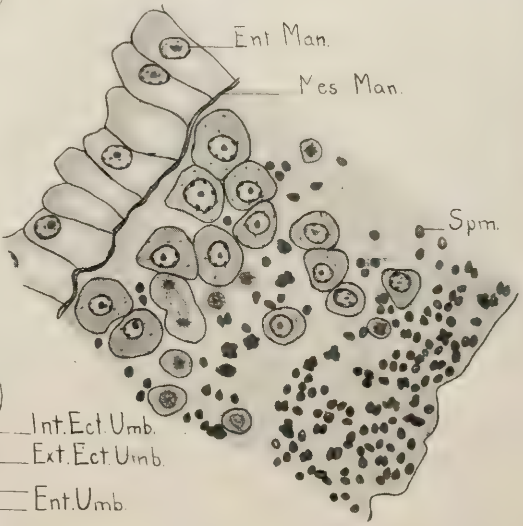
7


Ent. Man
Mes. Man.
S.C
E
S.C.

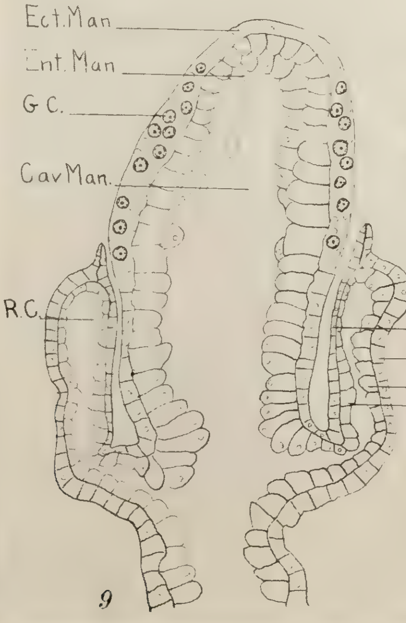
10


R.C.
Cav Man
GC
Ext Ect Umb
Ent Umb.
Int Ect Umb
Ect Man
Ent. Man

8


Ent Man.
Mes Man.
Spm.

11


Ect. Man
Ent. Man
G.C.
Cav Man.
R.C.
Int. Ect. Umb.
Ext. Ect. Umb.
Ent. Umb.

9

PLATE LIV.

FIG. 1. Tip of leaf showing venation, × 66: *a*, group of tracheids where veins end; *b*, free endings of veinlets.

FIG. 2. Venation farther back from the apex, × 66.

FIG. 3. Detailed drawing of group of tracheids at end of vein, × 275.

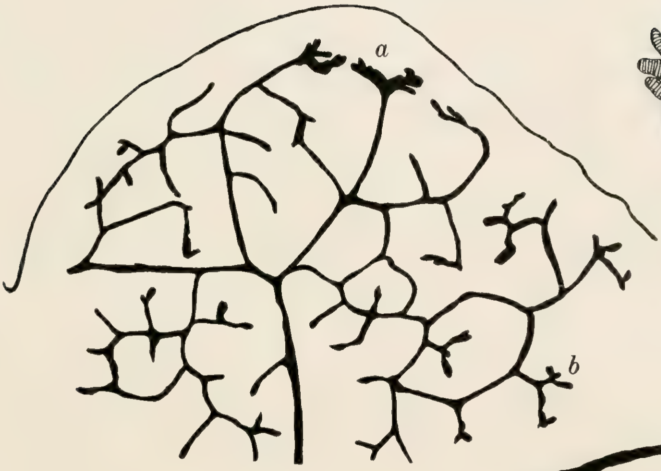
FIG. 4. Stellate hair from epidermis of leaf, × 175.

FIG. 5. Cross section of stoma of leaf, × 375: *a*, air chamber.

FIG. 6. Cross section lateral view of stoma.

FIG. 7. Epidermis of stem showing stomata, × 275.

PLATE LIV.



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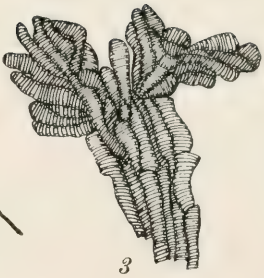

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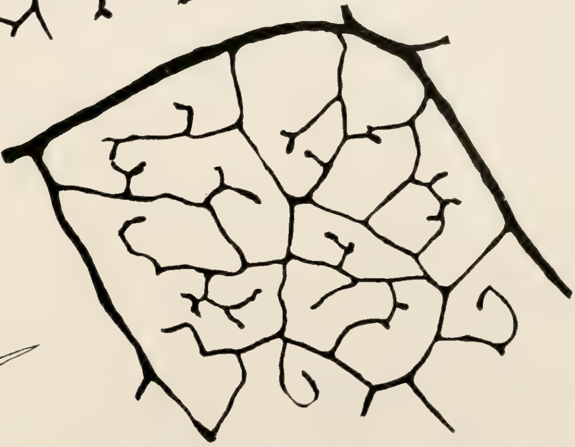

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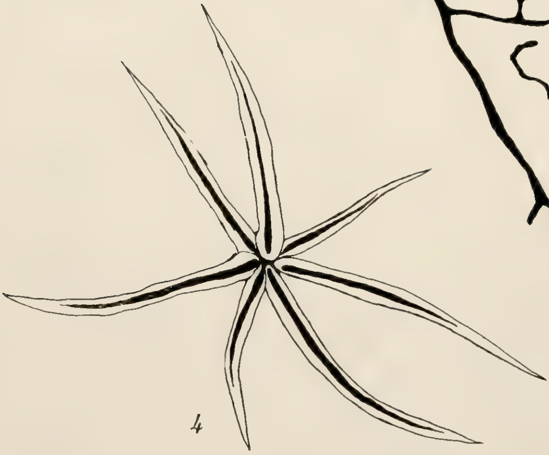

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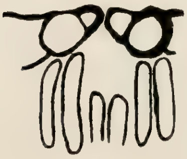

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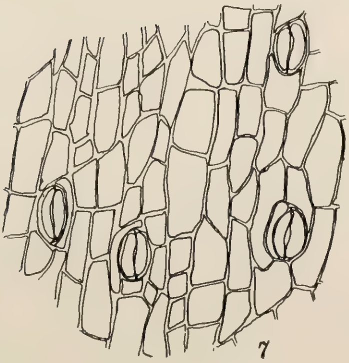

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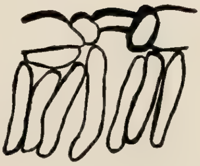


6

PLATE LV.

FIG. 8. Cross section of leaf showing part of a large vein, × 375: *a*, epidermal cells; *b*, mucilagenous cell; *c*, palisade cell; *d*, chloroplasts; *e*, tracheids; *f*, glandular hair; *g*, long border parenchyma cells surrounding the veins.

FIG. 9. Cross section of a large vein, × 375: *a*, vascular bundle; *b*, border parenchyma cell; *c*, mucilagenous modification of cell walls.

FIG. 10. Cross section of leaf, × 275; *a*, palisade cells; *b*, small vein; *c*, stoma in cross section; *d*, longitudinal section through a stoma.

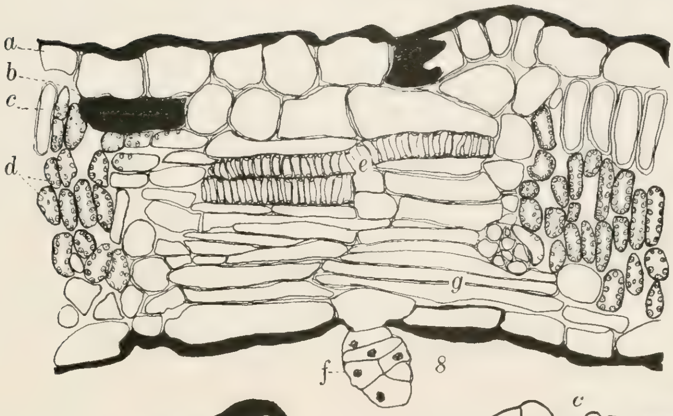
FIG. 11. Tangential section of leaf, × 275: *a*, vein; *b*, ending of vein; *c*, palisade cells.

FIG. 12. Tangential section through palisade cells, × 275.

FIG. 13. Tangential section showing epidermis, × 275: *a*, stoma; *b*, mucilagenous cell.

FIG. 14. Tangential section showing large border parenchyma cells beneath vein, × 275.

PLATE LV.



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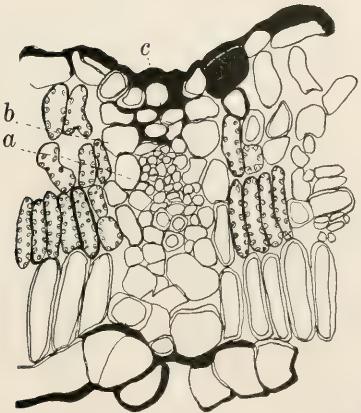

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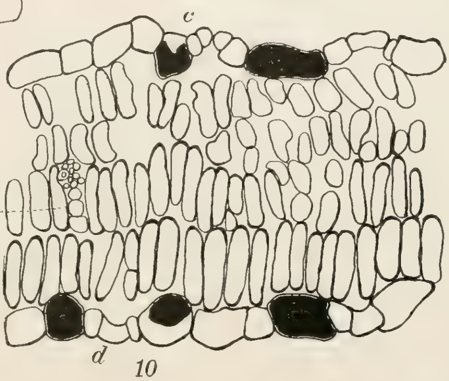

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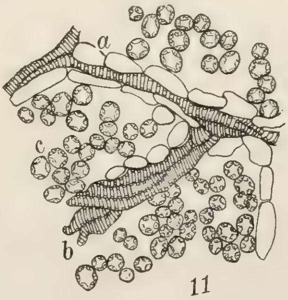

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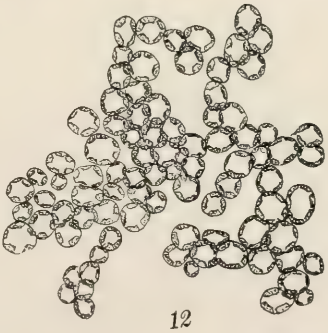

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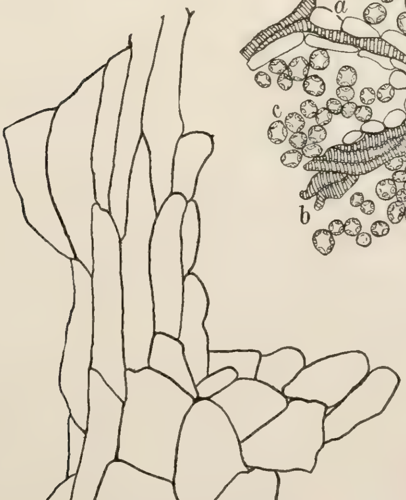

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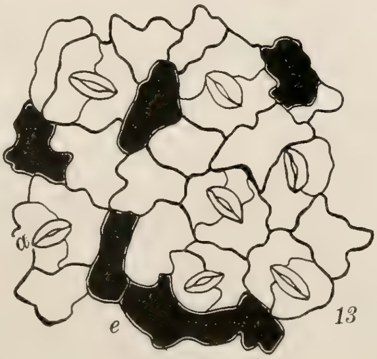


13

PLATE LVI.

FIG. 15. Cross section of stem showing distribution of mucilage, × 75: *m*, mucilage cells.

FIG. 16. Oblique section of stem showing distribution of mucilage, × 70: *e*, pocket where mucilage has dropped out; *d*, pocket containing mucilage.

FIG. 17. Cross section of stem showing distribution of crystals of calcium oxalate at *a*, × 20.

FIG. 18. Wood fiber, × 120.

FIG. 19. Crystals of calcium oxalate, × 375.

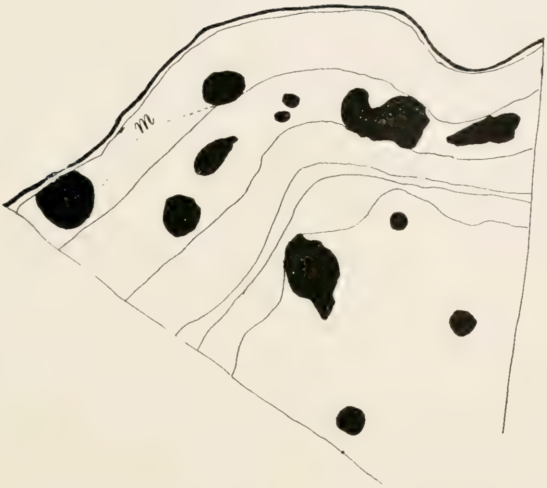
FIG. 20. Starch grains in stem, × 375.

FIG. 21. Starch grains in root, × 375.

FIG. 22. Cross section of petiole of leaf, × 70: *a*, vascular bundle; *b*, parenchyma of the cortex; *c*, collenchyma; *d*, glandular hair; *e*, epidermis.

FIG. 23. Vascular bundle of petiole, × 275: *a*, phloëm; *b*, tracheal tubes.

PLATE LVI.



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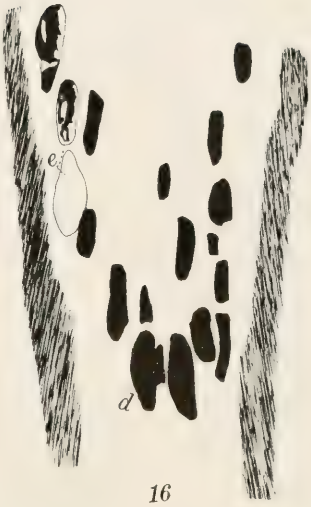

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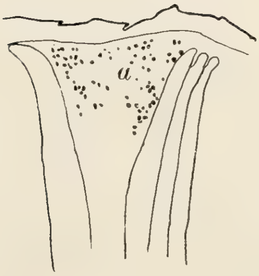

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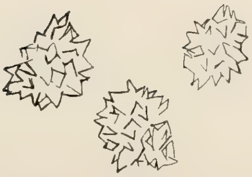

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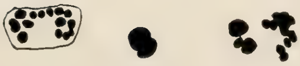

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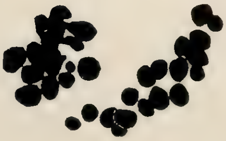

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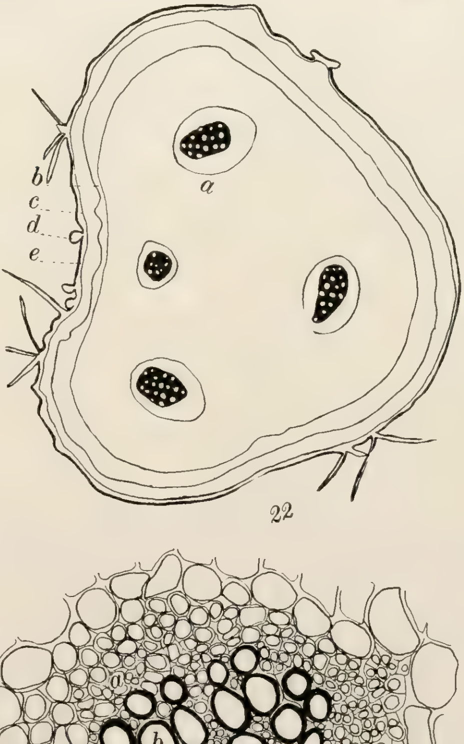

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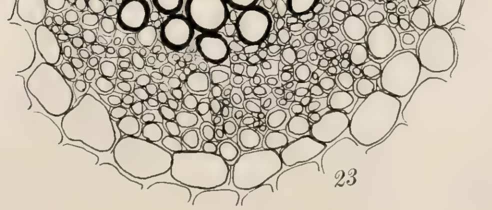

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PLATE LVII.

FIG. 24. Cross section of young stem, × 160: *a*, greatly swollen mucilage cavity; *b*, collenchyma; *c*, parenchyma of the cortex.

FIG. 25. Cross section of mid part of stem, × 160: *a*, mucilage cell.

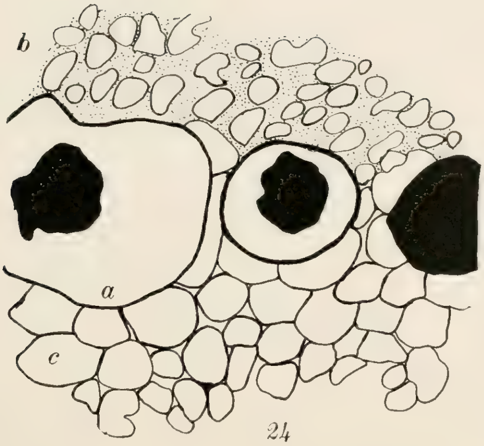
FIG. 26. Cross section of base of stem, × 160: *a*, mucilage cell.

FIG. 27. Cross section of old part of stem showing distribution of mucilage, × 25: *a*, collenchyma; *b*, parenchyma; *c*, bast fibers; *d*, phloëm; *e*, pith; *m*, mucilage.

FIG. 28. Cross section of young stem, × 25: *a*, collenchyma; *b*, parenchyma; *c*, bast fibers; *d*, phloëm; *e*, pith; *m*, mucilage.

FIG. 29. Cross section of a part of the root showing distribution of mucilage, × 25: *m*, mucilage.

PLATE LVII.



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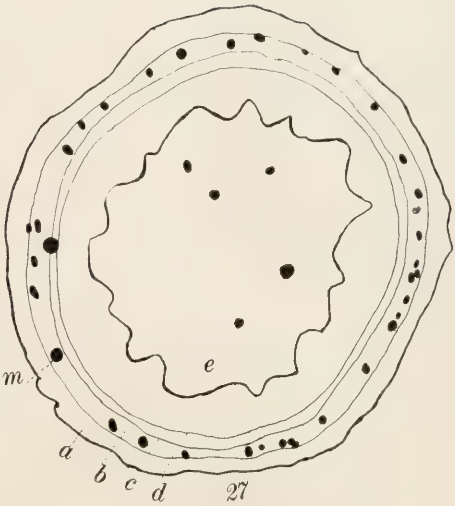

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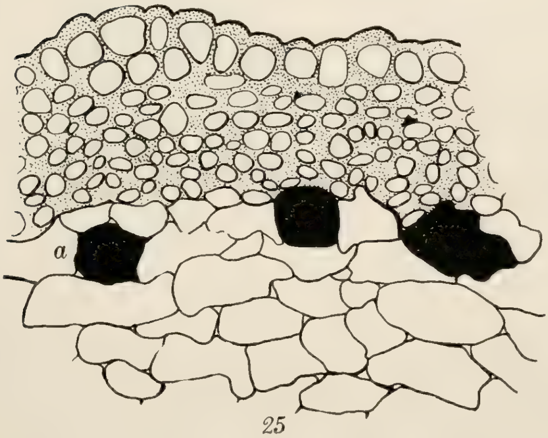

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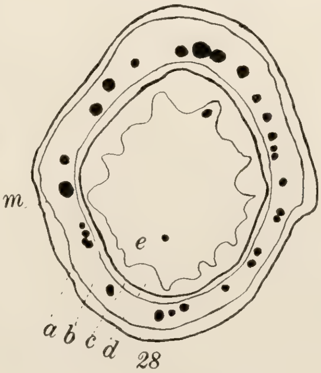

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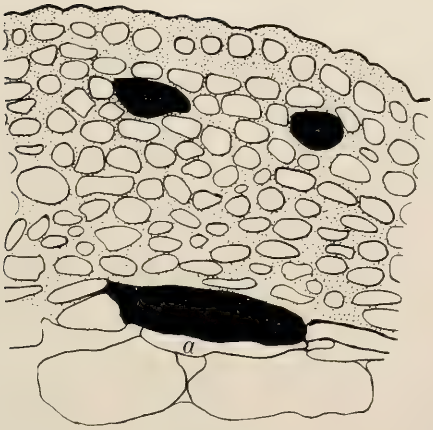

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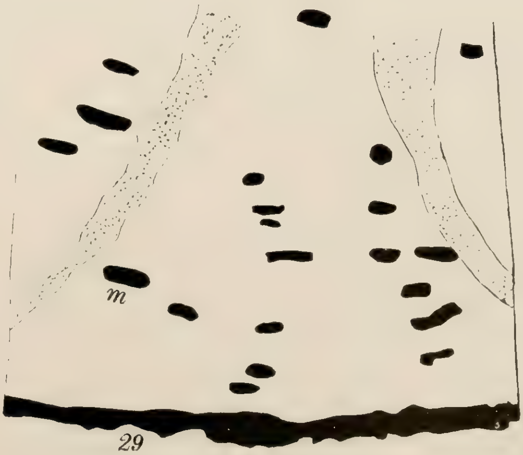


29

PLATE LVIII.

FIG. 30. Cross section of very young stem, × 25: *a*, multiple epidermis; *a′*, collenchyma; *b*, parenchyma; *c*, starch sheath; *d*, phloëm; *e*, xylem; *f*, pith.

FIG. 31. Cross section of a slightly older part of the same stem, × 25. Index same as for fig. 30.

FIG. 32. Section of same stem slightly older, × 25. Index same as for fig. 30, except *i*, bast fibers.

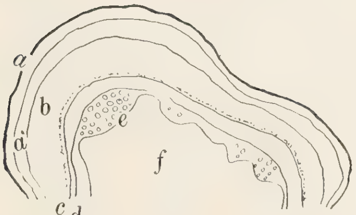
FIG. 33. Older section of the same stem, × 25. Index the same as for fig. 32.

FIG. 34. Older section of stem, × 25: *a*, cork tissue; *b*, starch sheath; *c*, original bast fiber bundle; *d*, bast produced by the cambium; *f*, water tubes; *g*, pith.

FIG. 35. Older part of stem, × 25. Index same as for fig. 34.

FIG. 36. Cross section of root, × 25: *a*, cork; *b*, parenchyma of the cortex; *c*, parenchyma of the pericycle and of the medullary rays, filled with starch; *d*, phloëm tissue; *f*, water-conducting tissue.

PLATE LVIII.



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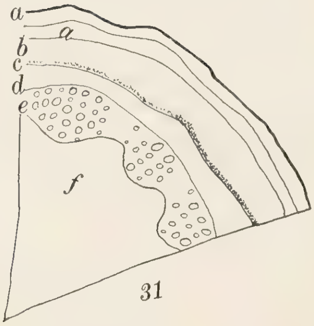

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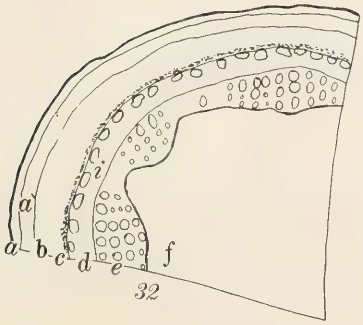

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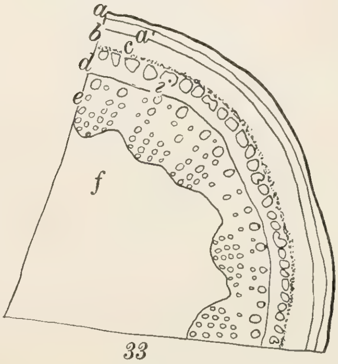

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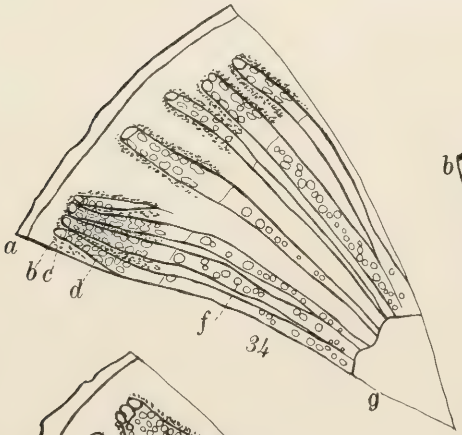

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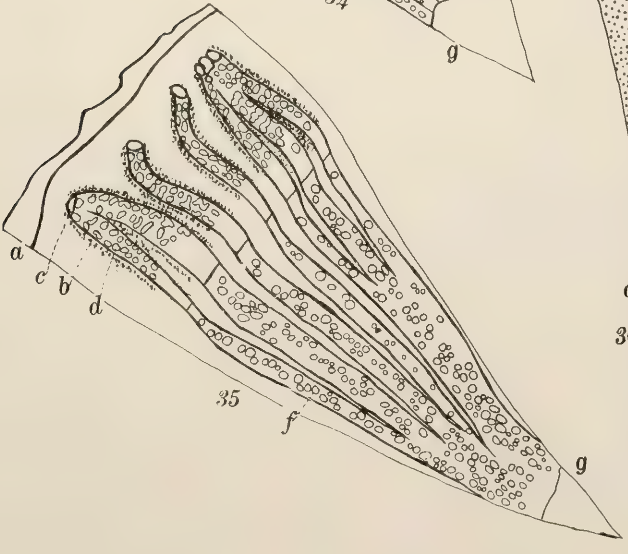

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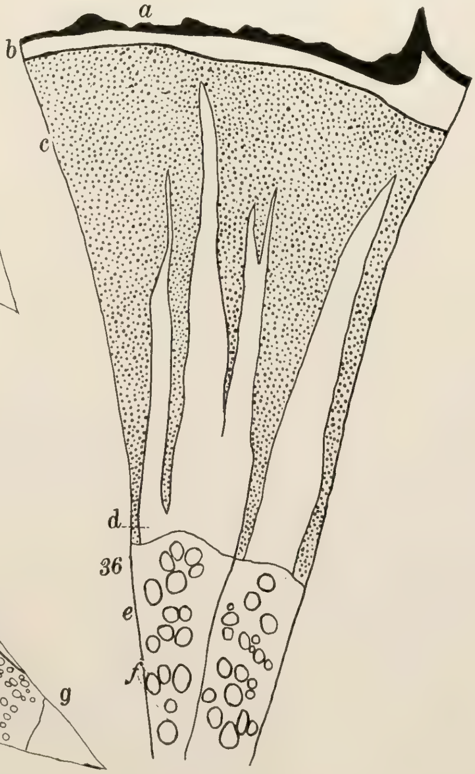
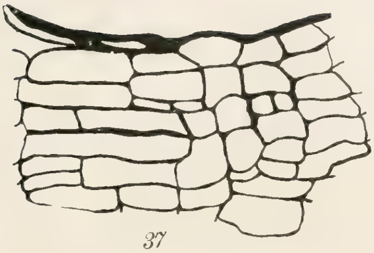


36

PLATE LIX.

FIG. 37. Cork tissue of the old stem, × 25.
FIG. 38. Original bast fiber bundle, × 25.
FIG. 39. Bast fiber bundle produced by cambium, × 25.
FIG. 40. Phloëm tissue, × 25.
FIG. 41. Water conducting tissue, × 25.
FIG. 42. Water tubes next to the pith, × 25.

PLATE LIX.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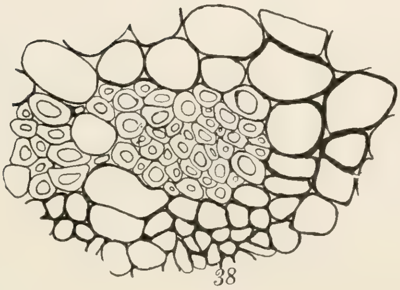
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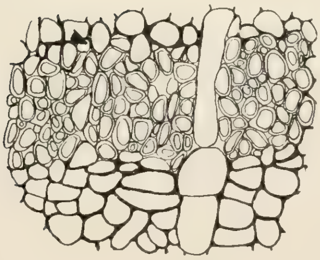
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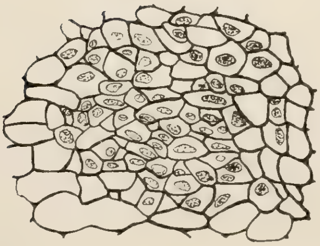
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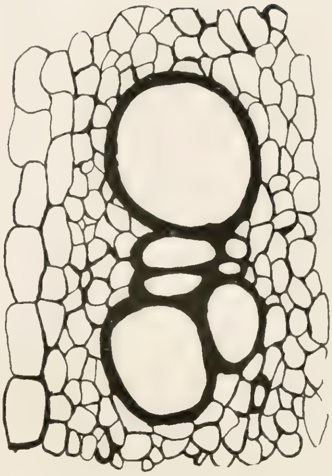
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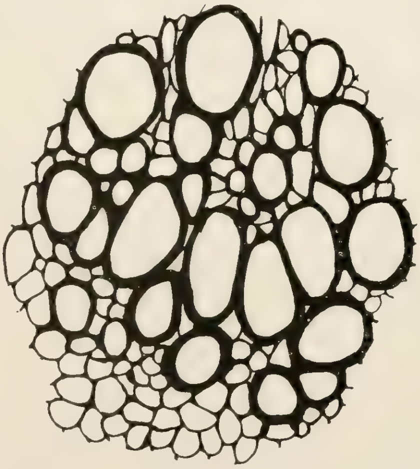
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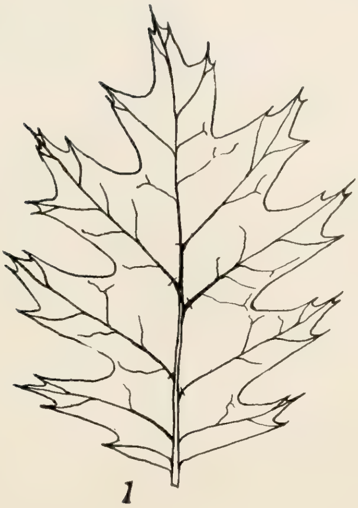
PLATE LX.

FIG. 1. Typical leaf of *Q. rubra*, × ½.
FIG. 2. Typical leaf of *Q. schneckii*, × ½.
FIG. 3. Typical leaf of *Q. coccinea*, × ½.
FIG. 4. Typical leaf of *Q. macrocarpa*, × ½.
FIG. 5. Upper epidermis and palisade cells of *Q. rubra*, × 275.
FIG. 6. Upper epidermis and palisade cells of *Q. schneckii*, × 275.

PLATE LX.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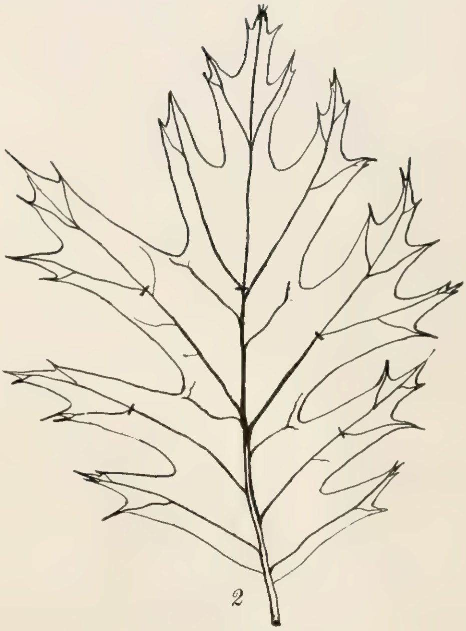
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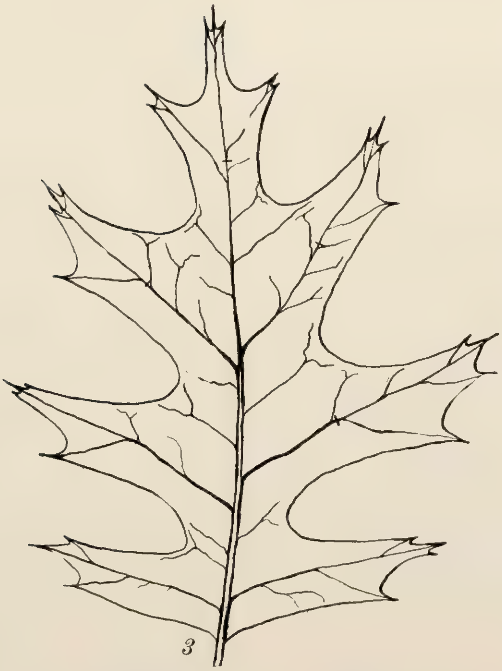
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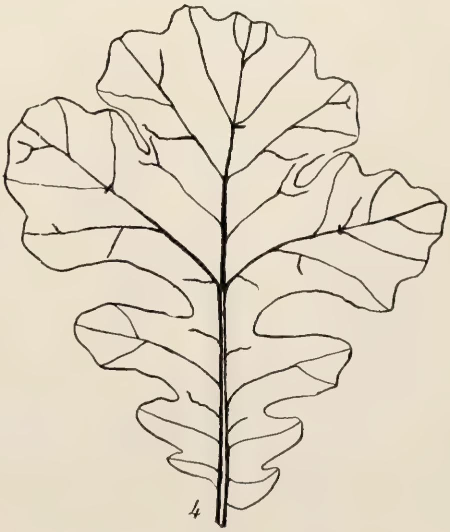
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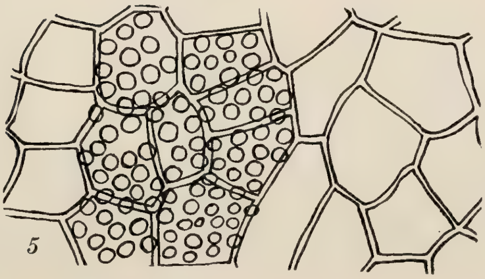
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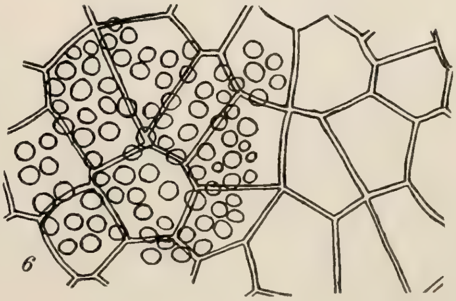
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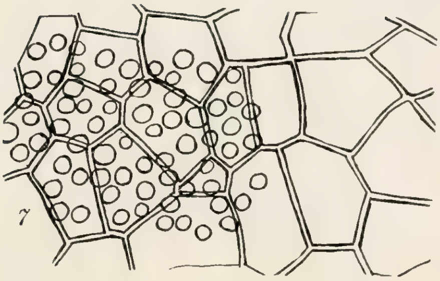
PLATE LXI.

FIG. 7. Upper epidermis and palisade cells of *Q. coccinea*, × 275.
FIG. 8. Upper epidermis and palisade cells of *Q. macrocarpa*, × 275.
FIG. 9. Lower epidermis of leaf of *Q. rubra*, × 275.
FIG. 10. Lower epidermis of leaf of *Q. schneckii*, × 275.
FIG. 11. Lower epidermis of leaf of *Q. coccinea*, × 275.
FIG. 12. Lower epidermis of leaf of *Q. macrocarpa*, × 275.
FIG. 13. Cross section of leaf of *Q. rubra*, × 235.
FIG. 14. Cross section of leaf of *Q. schneckii*, × 235.
FIG. 15. Cross section of leaf of *Q. coccinea*, × 235.
FIG. 16. Cross section of leaf of *Q. macrocarpa*, × 235.

PLATE LXI.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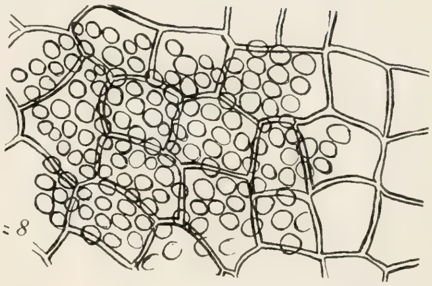
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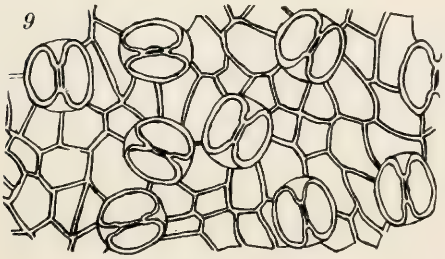
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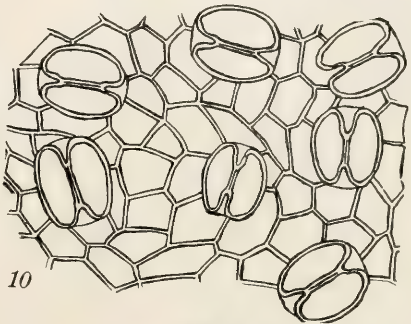
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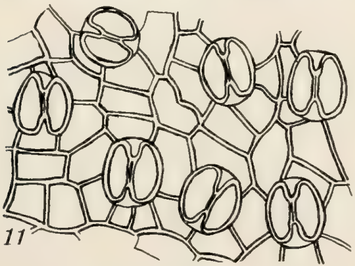
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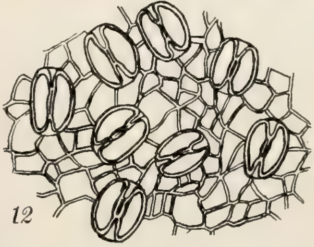
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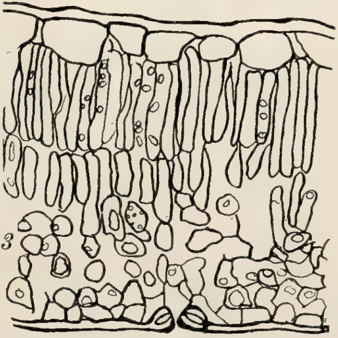
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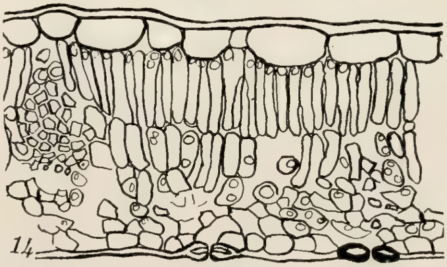
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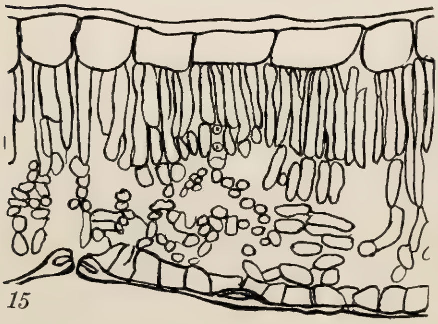
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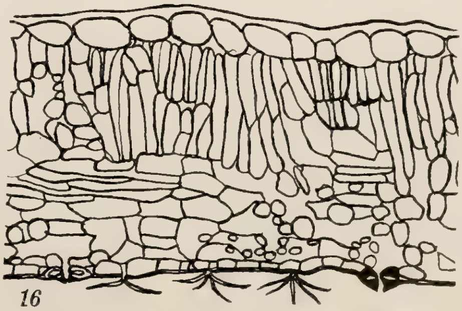

16

PLATE LXII.

FIGS. 17, 18, and 19. Cross section of midrib of *Q. rubra,* showing manner of ending of middle vascular bundle. Phloëm stippled.

FIGS. 20, 21, and 22. Cross section of midrib of *Q. coccinea,* showing manner of ending of middle vascular bundle. Phloëm stippled.

FIGS. 23, 24, and 25. Cross section of midrib of *Q. schneckii,* showing manner of ending of middle vascular bundle. Phloëm stippled.

FIGS. 26, 27, 28, 29, and 30. Cross section of midrib of *Q. macrocarpa,* showing manner of ending of middle vascular bundle. Phloëm stippled.

FIG. 31. Cross section of stem of *Q. rubra,* current year: *w,* pith; *x,* xylem; *y,* phloëm; *z,* bast. × 12.

FIG. 32. Cross section of stem of *Q. schneckii,* current year: *w, x, y,* and *z* same as in fig. 31. × 12.

FIG. 33. Cross section of stem of *Q. coccinea,* current year: *w, x, y,* and *z* same as in fig. 31. × 12.

FIG. 34. Cross section of stem of *Q. macrocarpa,* current year: *w, x, y,* and *z* same as in fig. 31. × 12.

FIG. 35. Wood fiber of *Q. macrocarpa.* × 55.

FIG. 36. Bast fiber of *Q. macrocarpa.* × 55.

FIG. 37. Wood fiber of *Q. rubra.* × 55.

FIG. 38. Bast fiber of *Q. rubra.* × 55.

FIG. 39. Wood fiber of *Q. schneckii.* × 55.

FIG. 40. Bast fiber of *Q. schneckii.* × 55.

FIG. 41. Wood fiber of *Q. coccinea.* × 55.

FIG. 42. Bast fiber of *Q. coccinea.* × 55.

PLATE LXII.

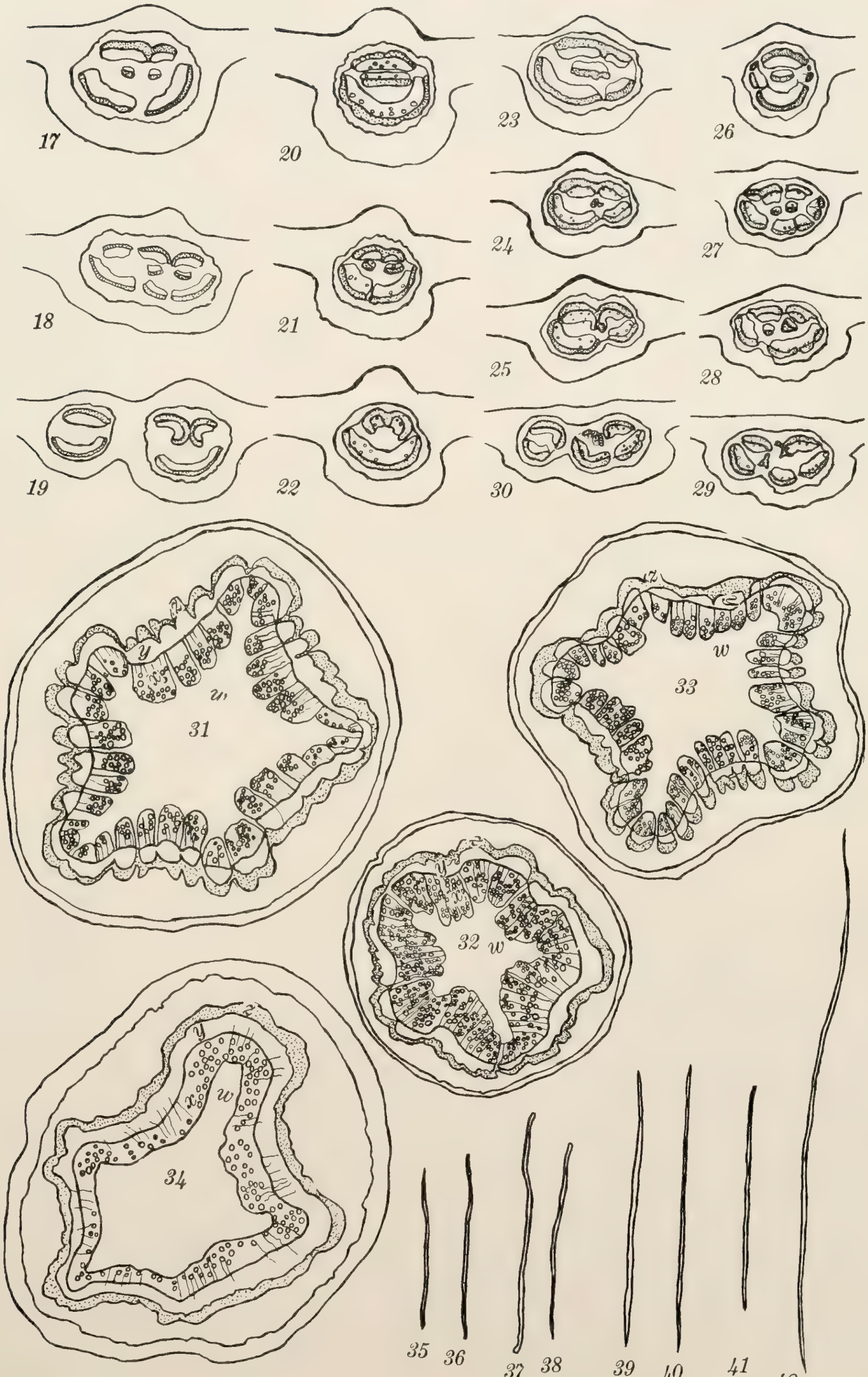

z
y
x
w
z
w
z
y
x
32 w
z
y
x
w

PLATE LXIII.

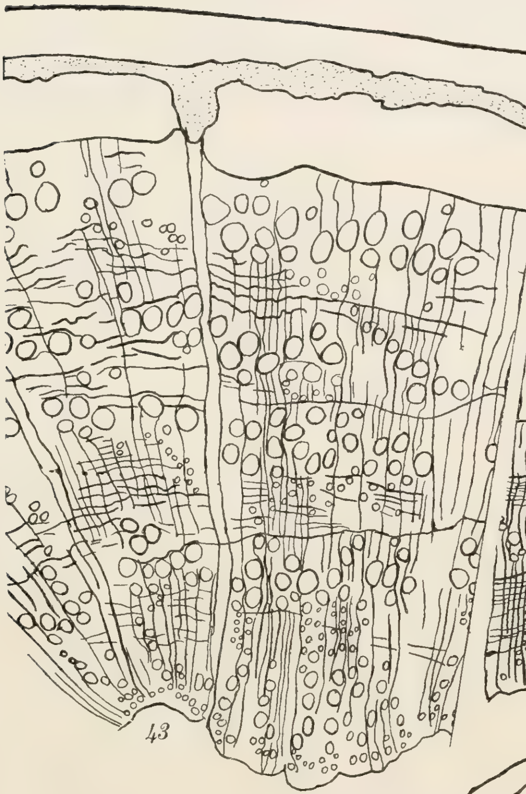
FIG. 43. Cross section of stem of *Q. schneckii,* in the fourth year, showing tracheal tubes, medullary rays, and bark. Bast stippled. × 25.

FIG. 44. Cross section of stem of *Q. macrocarpa,* showing the same elements. × 25.

FIG. 45. Cross section of stem of *Q. coccinea,* showing the same elements. × 25.

FIG. 46. Cross section of stem of *Q. rubra,* showing the same elements. × 25.

PLATE LXIII.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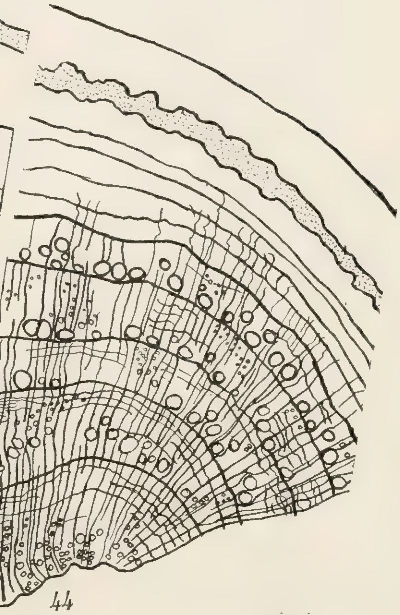
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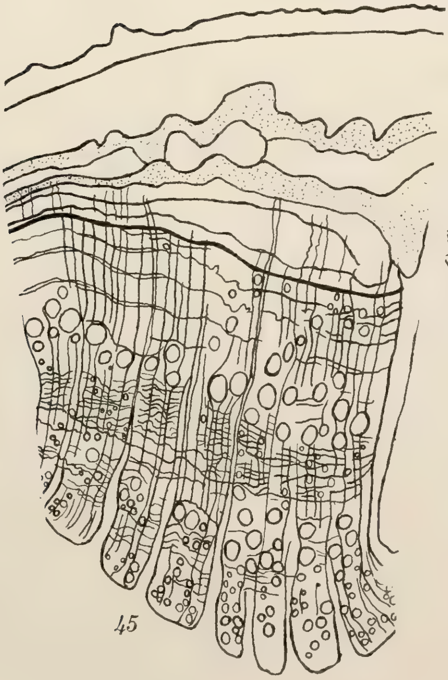
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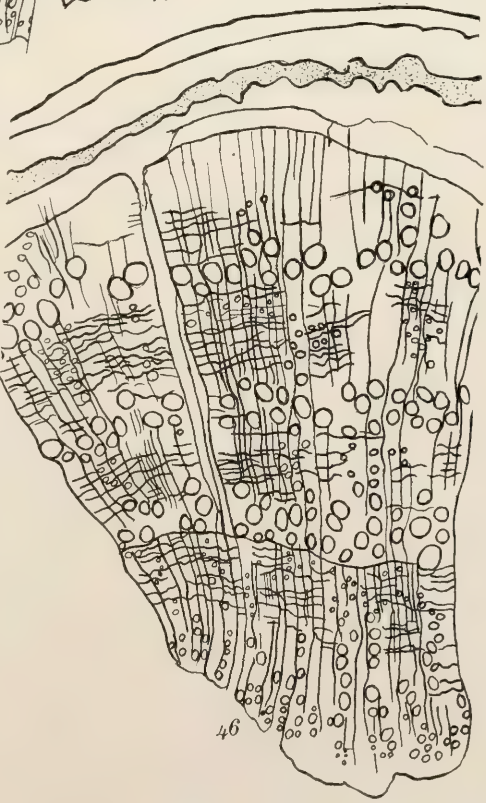

46

PLATE LXIV.

FIG. 47. Tangential section of stem of Q. *rubra*, showing the number and size of medullary rays. × 52.

FIG. 48. Tangential section, showing same for Q. *schneckii.* × 52.

FIG. 49. Tangential section, showing same for Q. *coccinea.* × 52.

FIG. 50. Tangential section, showing same for Q. *macrocarpa.* × 52.

FIG. 51. Acorn of Q. *rubra.* × ½.

FIG. 52. Acorn of Q. *schneckii.* × ½.

FIG. 53. Acorn of Q. *coccinea.* × ½.

FIG. 54. Acorn of Q. *macrocarpa.* × ½.

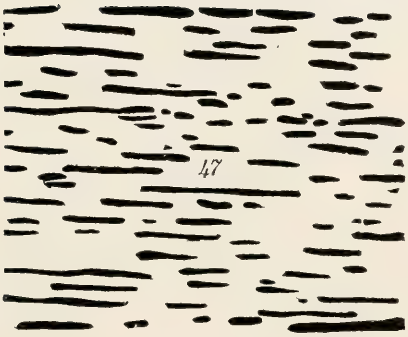
FIG. 55. Cross section of shell of Q. *rubra.* × 23.

FIG. 56. Cross section of shell of Q. *schneckii.* × 23.

FIG. 57. Cross section of shell of Q. *macrocarpa.* × 23.

FIG. 58. Cross section of shell of Q. *coccinea.* × 23.

PLATE LXIV.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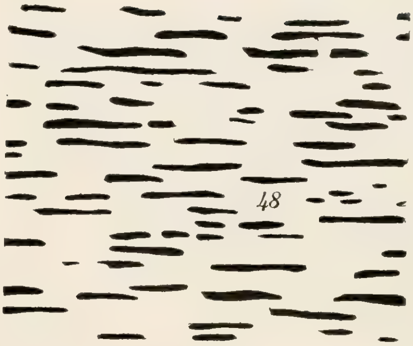
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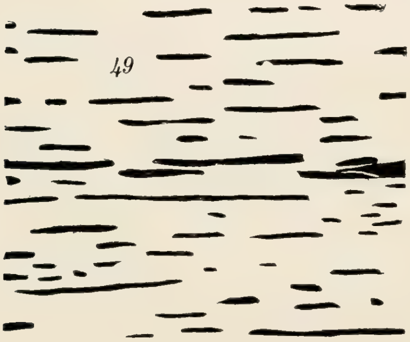
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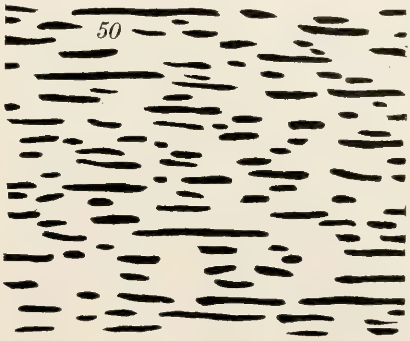
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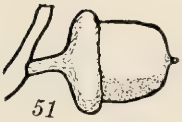
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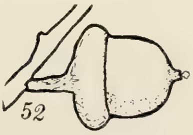
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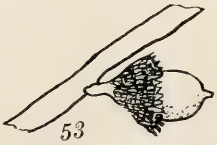
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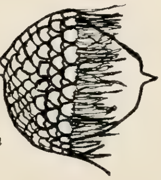
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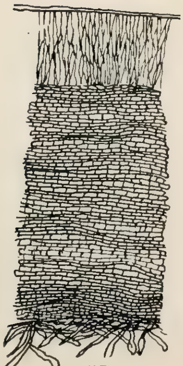
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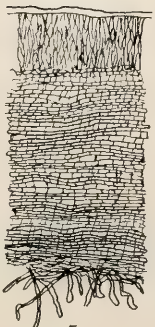
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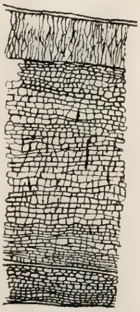
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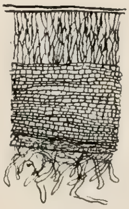

58

PLATE LXV.

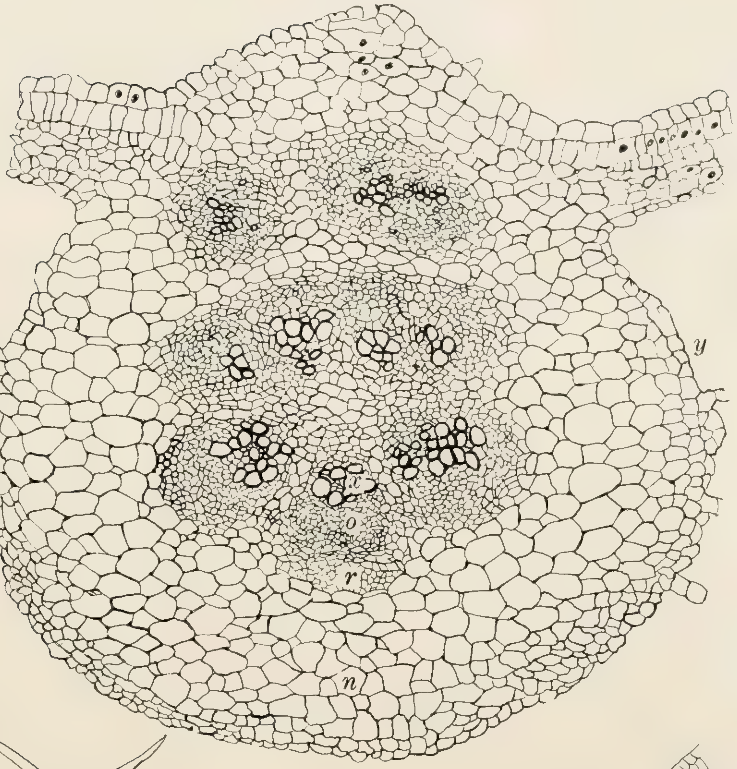
FIG. 1. Multicellular clothing hair of the epidermis of a very young leaf. × 110.

FIG. 2. Cross section of the midrib of a very young leaf: *m*, clothing hair; *a*, glandular hair; *y*, epidermis; *n*, parenchyma; *r*, cells which will develop into bast fibers; *o*, phloëm; *x*, tracheal tubes. × 110.

FIG. 3. Cross section of the midrib of a somewhat older leaf: *y*, epidermis; *n*, parenchyma; *r*, cells which will develop into bast fibers; *o*, phloëm; *x*, xylem. × 110.

FIG. 4. Cross section of the midrib of a mature leaf: *y*, epidermis; *n*, parenchyma; *r*, bast fibers; *o*, phloëm; *x*, xylem. × 110.

PLATE LXV.



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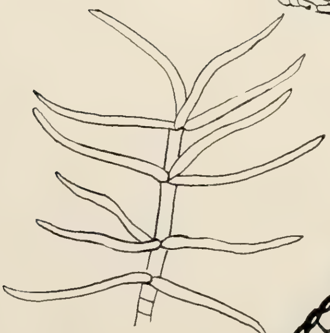

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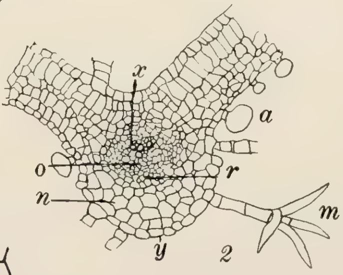

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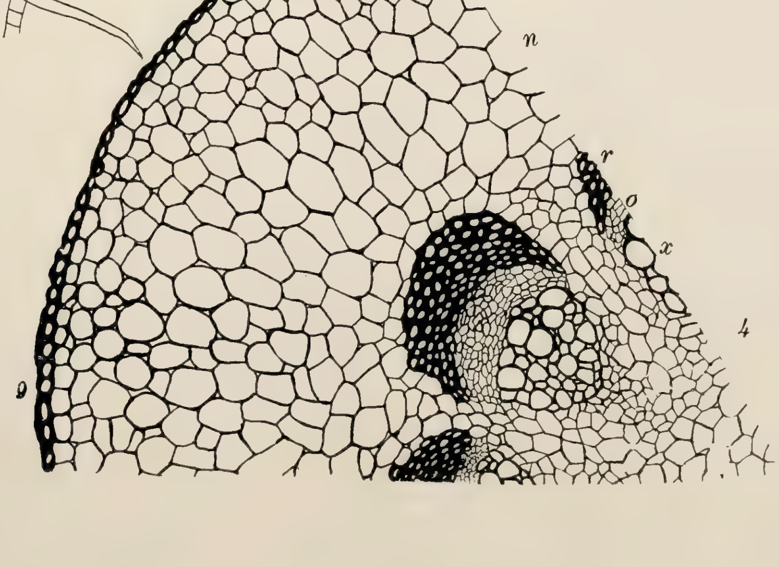


4

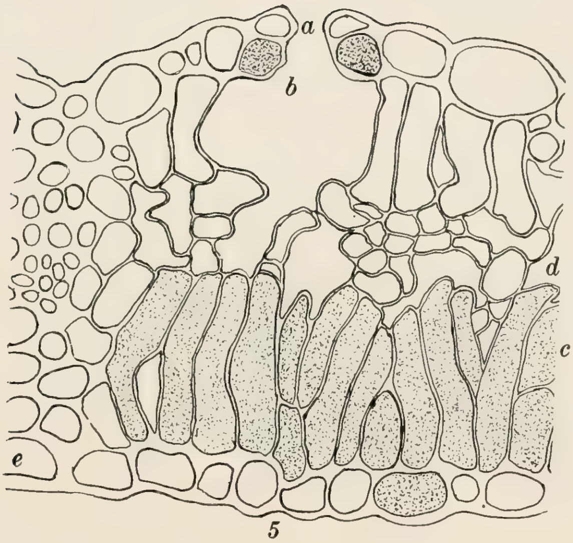
PLATE LXVI.

Fig. 5. Cross section of a leaf stained with methylene blue. Stippling indicates cells whose contents stained a deep blue: *a*, guard cells; *b*, epidermal cell beneath guard cell; *d*, spongy parenchyma; *c*, palisade cells; *e*, upper epidermis. × 400.

Fig. 6. Lower epidermis of leaf: *a*, guard cell; *b*, epidermal cell beneath guard cell whose contents took a deep stain with methylene blue. × 400.

Fig. 7. Surface view of a bleached leaf embracing the epidermal, the palisade, and the vascular bundle systems. × 183.

PLATE LXVI.



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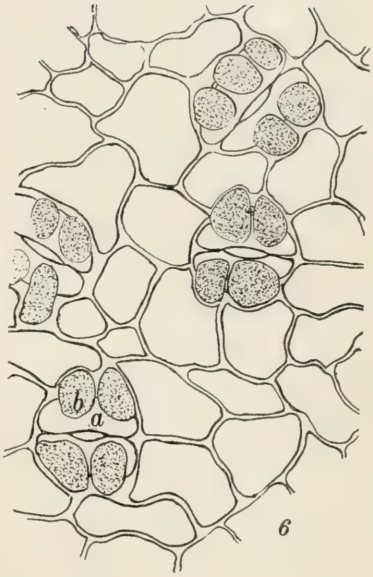

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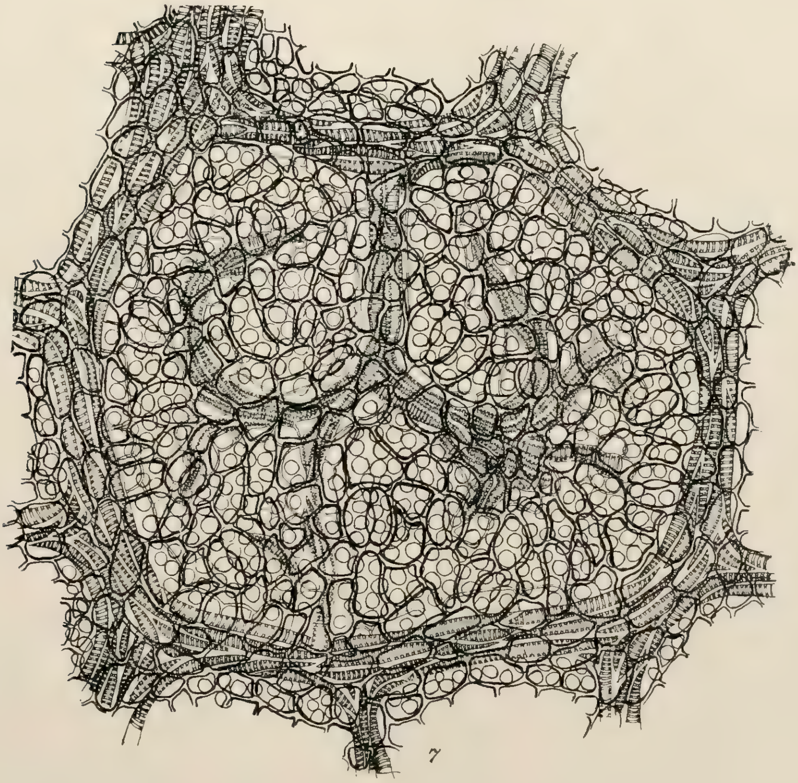
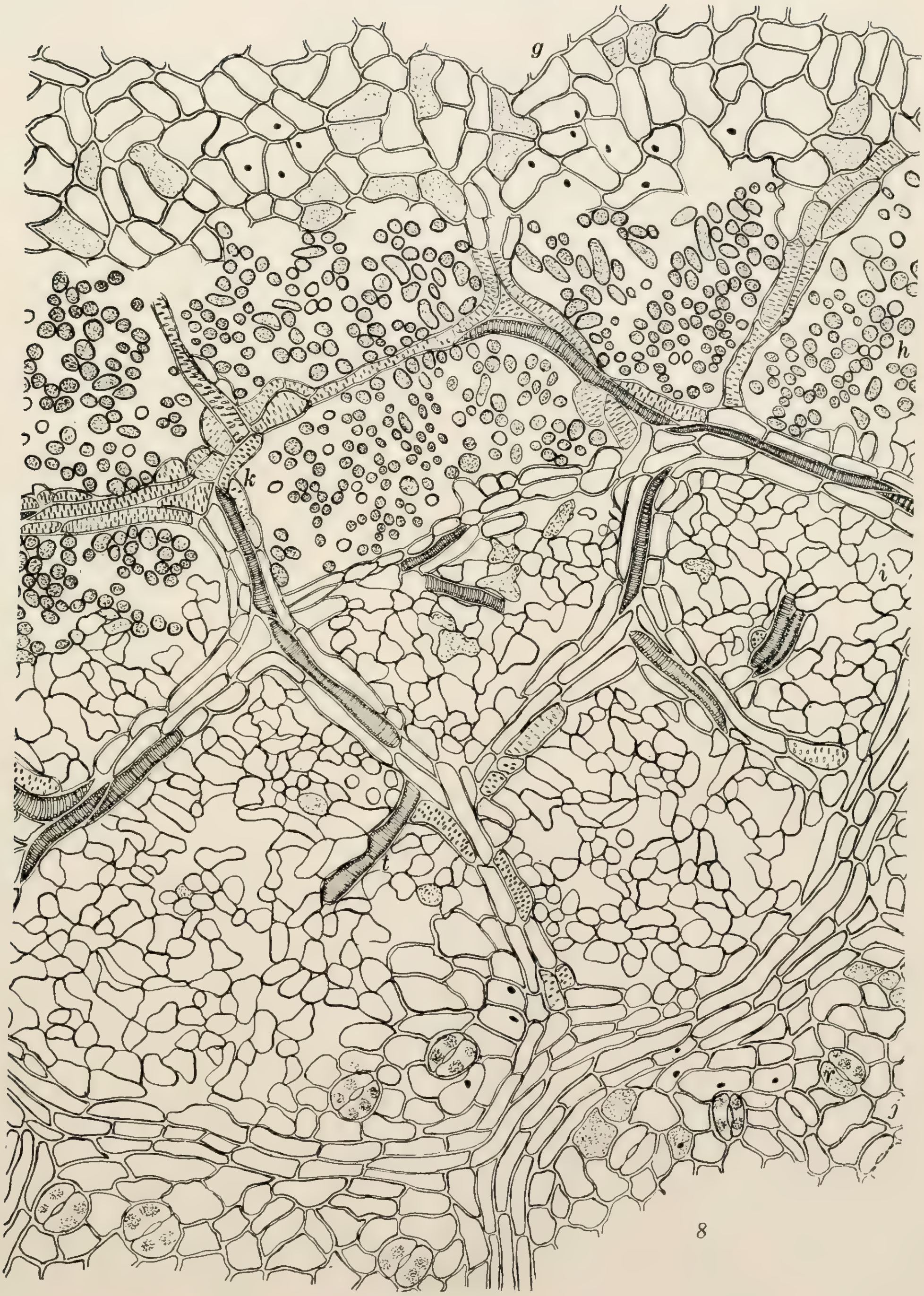


7

PLATE LXVII.

FIG. 8. An oblique section of a leaf nearly parallel to the surface and embracing zones from surface to surface. Stained with methylene blue. Stippling indicates cells whose contents were deeply stained: *g*, upper epidermis; *h*, palisade cells; *k*, border parenchyma; *t*, tracheid with annular thickenings; *j*, lower epidermis; *r*, stoma. × 187.

PLATE LXVII.



8

PLATE LXVIII.

FIGS. 9 to 19, inclusive, are cross sections of the midrib of a leaf, the sections being cut at intervals from the tip to the base of the leaf blade. The xylem and bast fibers are colered black in figures 9 to 29, inclusive. × 20.

FIG. 19, *b*, bast fibers; *x*, xylem.

FIG. 20. Cross section of the petiole of a leaf just below the base of the leaf blade, showing two rings of vascular bundles. × 20.

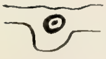
FIG. 21. Cross section of petiole. This diagram represents the typical arrangement of the vascular bundles in the petiole. × 20.

FIG. 22. Cross section of petiole a short distance above the base. × 40.

FIG. 23. Cross section of petiole just above the hollow in which the axillary bud lies. × 20.

FIG. 24. Cross section of petiole, showing the hollow, *h*, in which the axillary bud lies. × 20.

PLATE LXVIII.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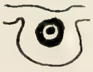
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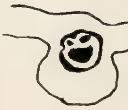
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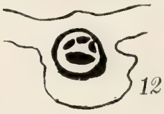
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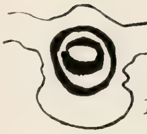
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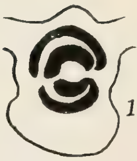
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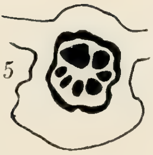
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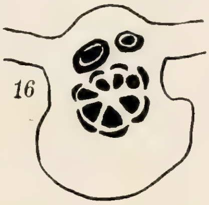
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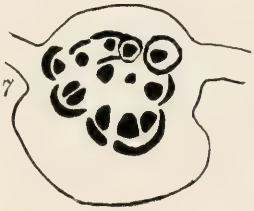
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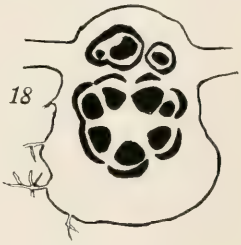
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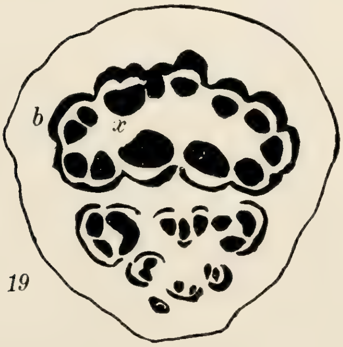

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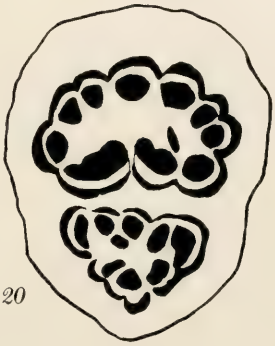
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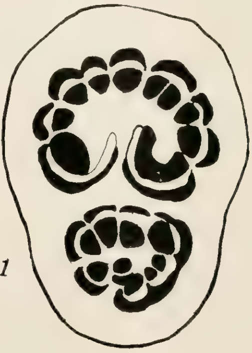
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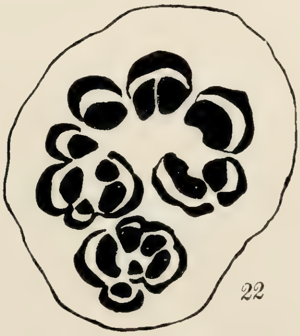
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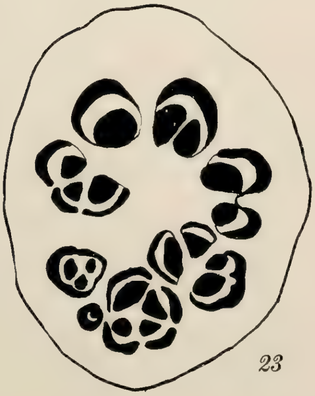
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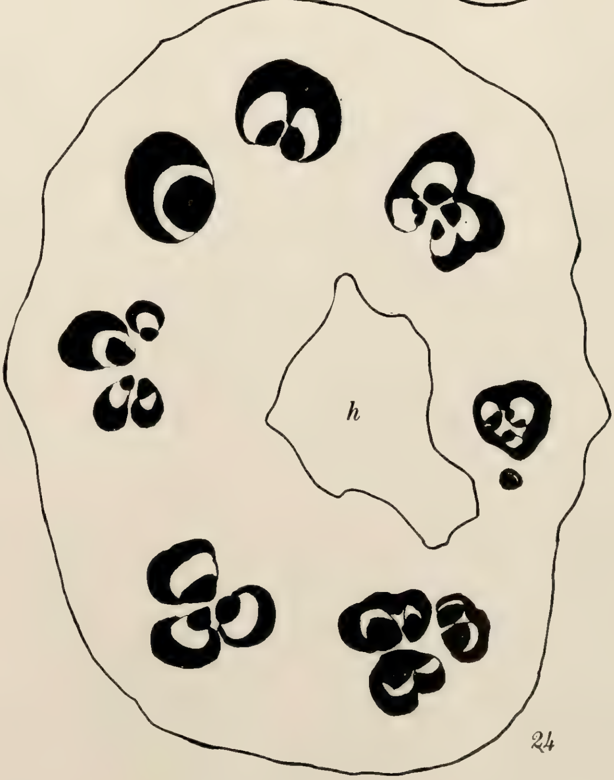

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PLATE LXIX.

FIGS. 25 to 29. A series of cross sections of the stem proceeding downward. Stippled region, *w*, indicates stone cells.

FIG. 25. Section just below terminal bud showing strands of leaf trace, *t*, *u*, *v*, *w*, *x*, *y*, and *z*, just before they enter the vascular ring of the stem. × 8.5.

FIG. 26. *t* and *z* are taking their places in the vascular rings of the stem; *w* is about to enter. × 8.5.

FIG. 27. *t*, *z*, and *w* are in the vascular ring of the stem. The other strands are approaching the ring. × 8.5.

FIG. 28. *t*, *v*, *w*, *x*, and *z* have now entered the vascular ring of the stem. Strands *u* and *y* are almost within the leaf trace gaps. × 8.5.

FIG. 29. All the leaf trace strands have occupied the leaf trace gaps. × 8.5.

FIG. 30. Twig showing terminal bud and lateral bud. × 1.25.

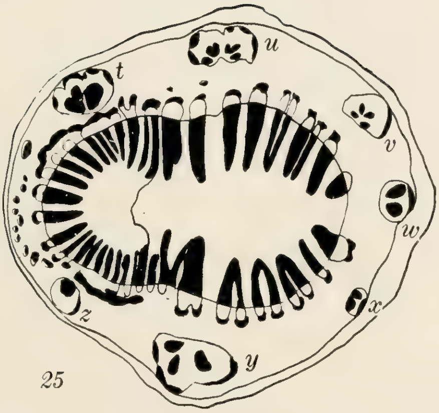
FIG. 31. Longitudinal section of terminal bud: *s*, outer scale; *t*, second bud scale covered with hairs and resin; *u*, third bud scale, and *v*, hairs growing from this scale; *w*, inflorescence; *x*, section through leaf. × 3.5.

FIG. 32. Cross section of bud through inflorescence. Lettering same as in fig. 31. × 3.5.

FIG. 33. Cross section of bud below inflorescence; *z*, peduncle. The other letters as in figs. 31 and 32. × 3.5.

FIG. 34. Dissection of bud: *a*, outer bud scale; *b*, second bud scale; *c*, bud after the two outer bud scales have been removed showing leaf, *s*, outside the third bud scale; *d*, leaf outside the third bud scale; *f*, *g*, *h*, and *i*, leaves in order from the outside in; *j*, inflorescence. × 3.5.

PLATE LXIX.



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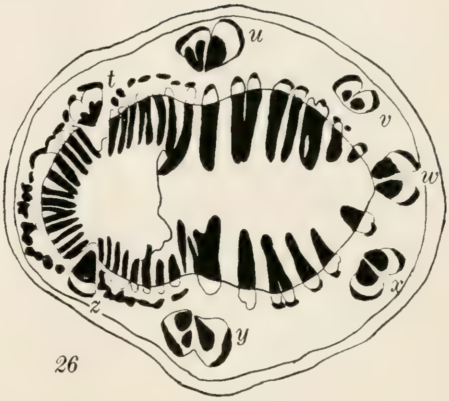

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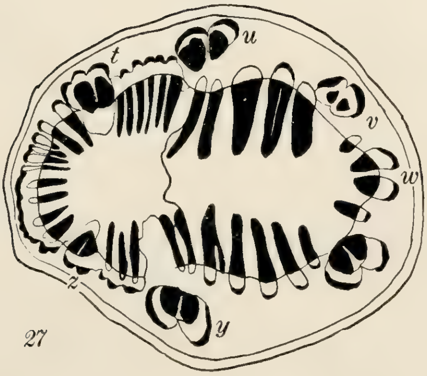

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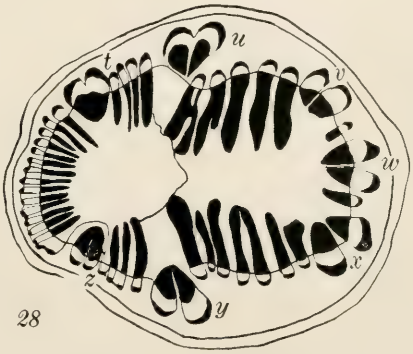

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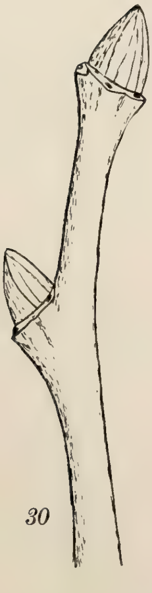

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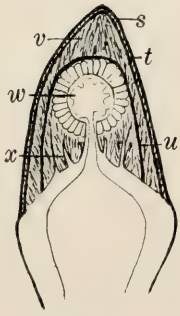

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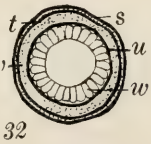

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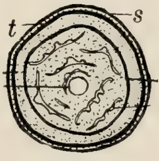

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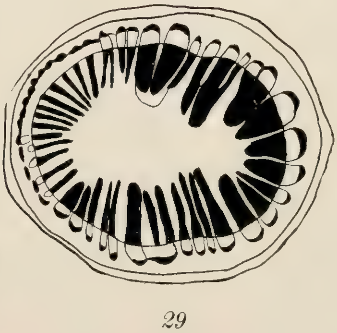

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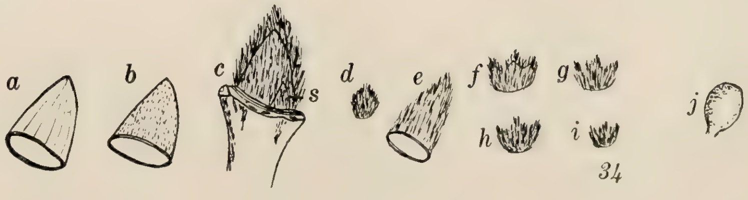
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j

34

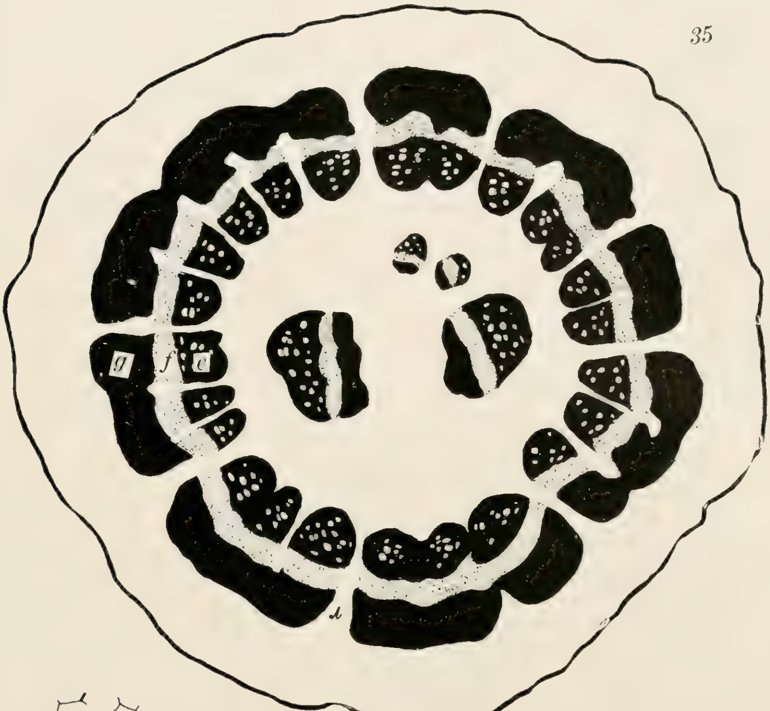
PLATE LXX.

FIG. 35. Cross-section of the peduncle: *g*, bast fibers; *f*, phloëm; *e*, xylem; *r*, medullary ray. × 47.

FIG. 36. Cross-section of peduncle: *d*, pith; *e*, phloëm; *f*, xylem; *h*, pith with thickened walls; *i*, xylem; *j*, phloëm; *k*, bast fibers; *l*, parenchyma; *m*, epidermis. × 158.5.

PLATE LXX.

35



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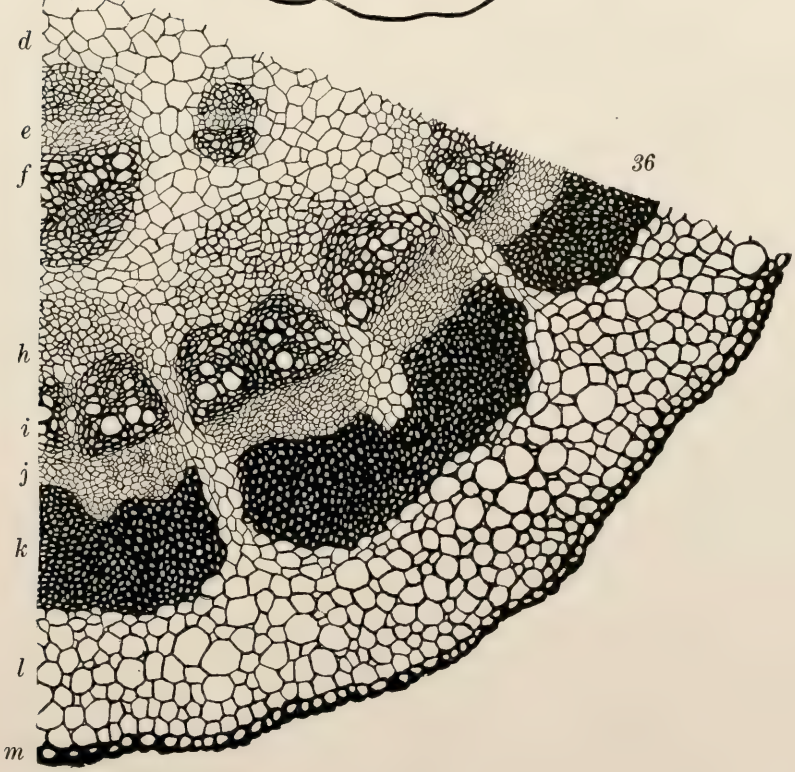


PLATE LXXI.

FIGS. 37 to 42, inclusive. A series of cross sections of the stem starting just above the base of the terminal bud and proceeding downward, showing how the rings of vascular bundles of three separate buds merge into the one ring of the stem which bears them. The xylem and bast fibers are colored black. × 13.5.

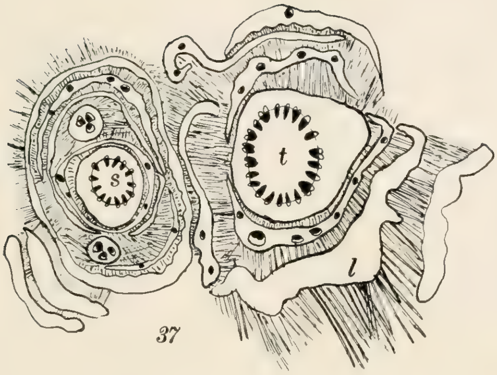
FIG. 37. Cross section showing the vascular bundles of two buds, *s* and *t*. The outer bud scales have been removed: *l*, leaf. × 13.5.

FIG. 38. *s* and *t* are closer together. There is stem tissue instead of leaves between them. × 13.5.

FIG. 39. *s* and *n* are approaching *t*. × 13.5.

FIG. 40. The ring, *t*, is opening to receive *s* and *n*. × 13.5.

PLATE LXXI.



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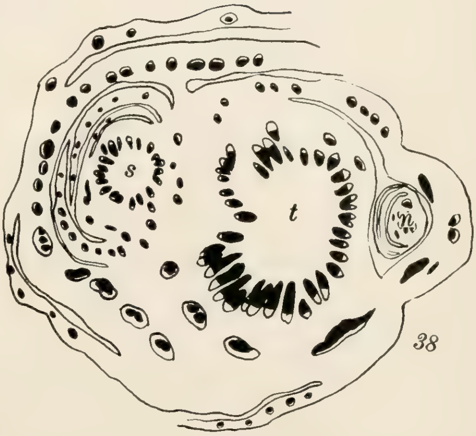

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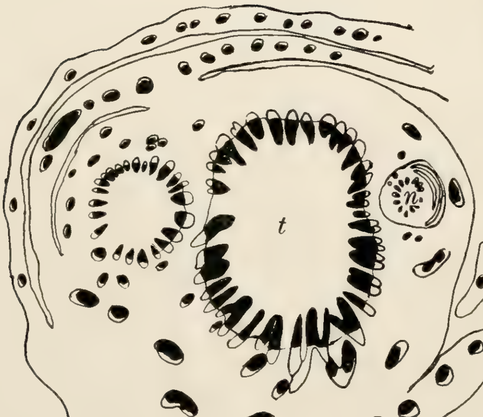

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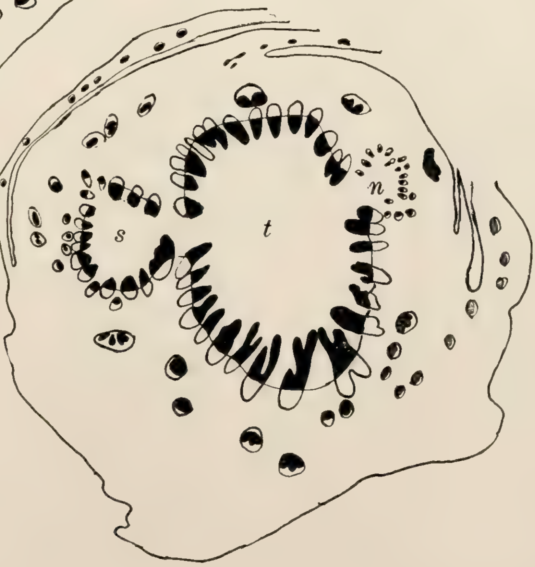

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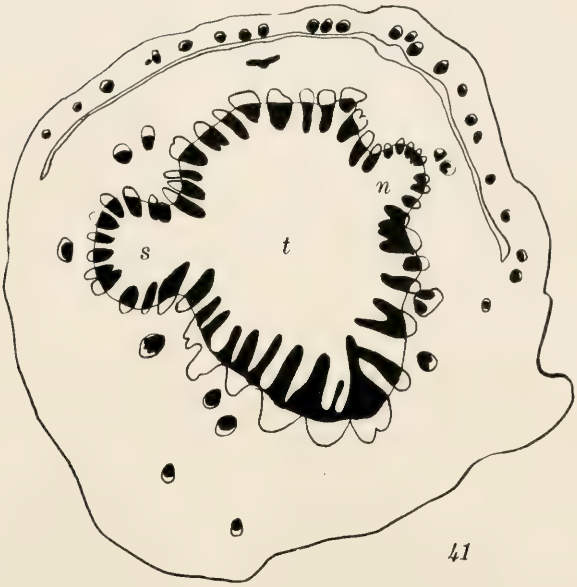
PLATE LXXII.

FIG. 41. *s*, *t*, and *n* are forming one ring. × 13.5.
FIG. 42. *s*, *t*, and *n* have merged into one ring. × 13.5.

PLATE LXXII.



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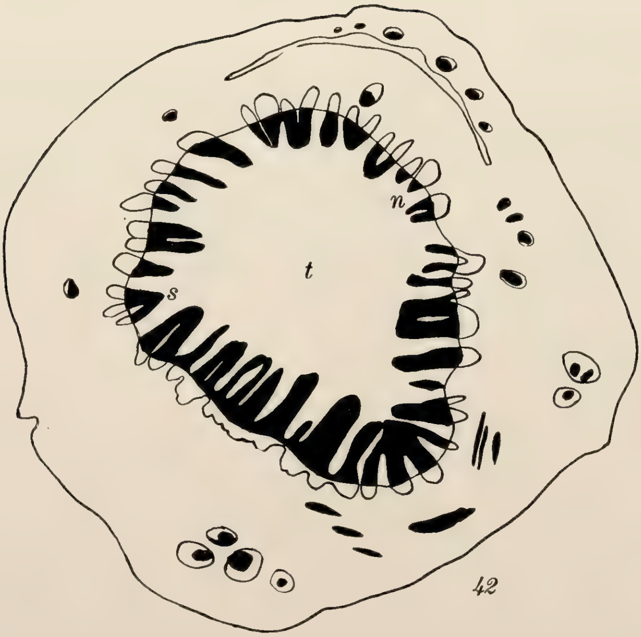

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PLATE LXXIII.

FIG. 43. Cross section of the bark of a stem five years old: *c*, cork; *b*, bast fibers; *l*, lignified tissue with the exception of the bast fibers; *u*, unlignified tissue. × 41.5.

FIG. 44. Cross section of bark: *c*, cork; *j*, parenchyma; *b*, bast fibers; *p*, phloëm; *m*, medullary ray. × 100.

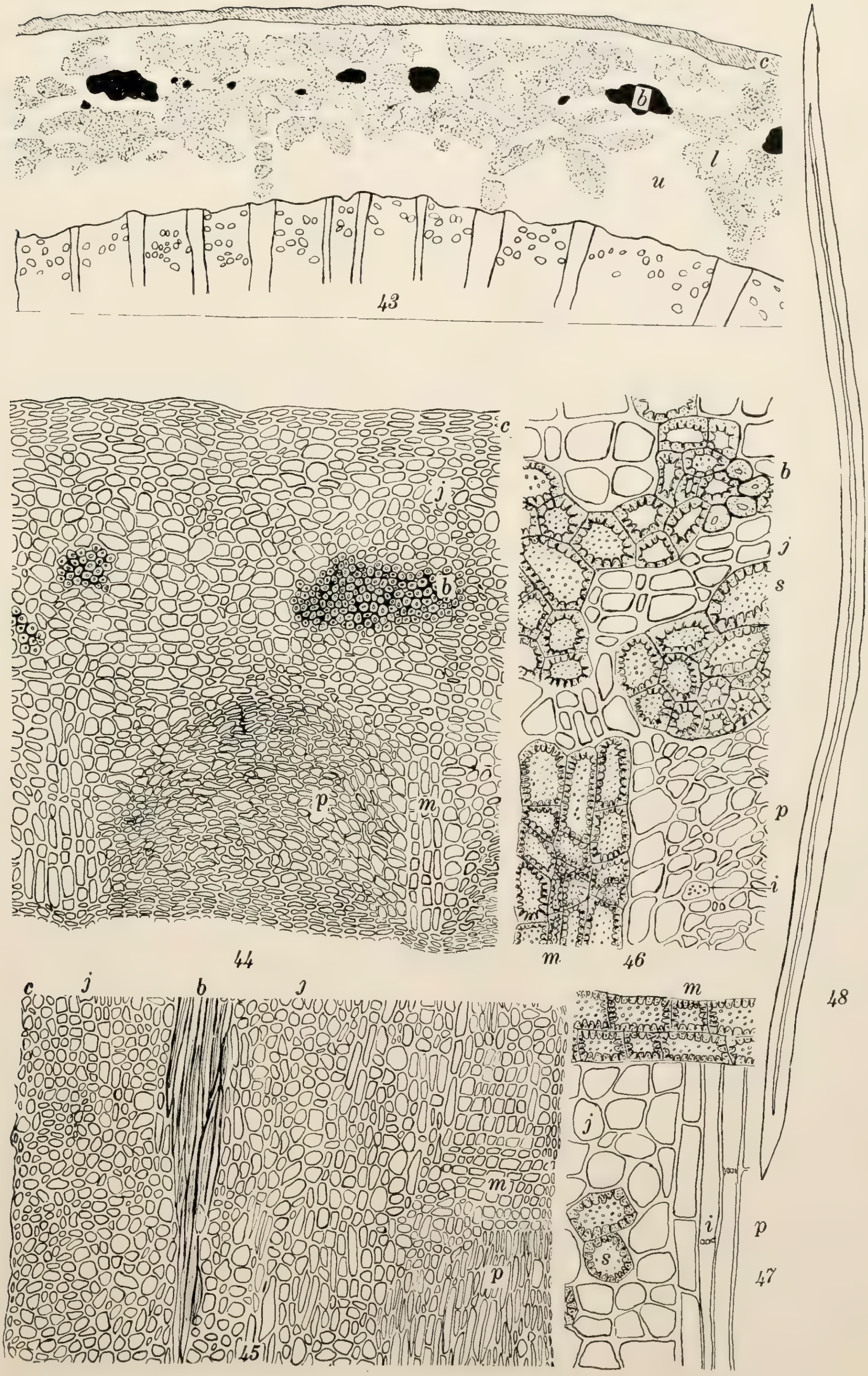
FIG. 45. Longitudinal section of the bark: *c*, cork; *j*, parenchyma; *b*, bast fibers; *m*, medullary rays; *p*, phloëm. × 100.

FIG. 46. Cross section of bark: *b*, bast fibers; *j*, parenchyma; *s*, stone cells; *p*, phloëm; *i*, sieve plate. Stippling indicates lignified tissue. × 277.5.

FIG. 47. Longitudinal section of the bark. Lettering same as in fig. 46. × 555.

FIG. 48. Bast fiber of medium length from the bark. × 277.5.

PLATE LXXIII.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c
j
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44
b
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s
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i
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46
48
c
j
b
j
m
p
45
m
j
s
i
p
47

PLATE LXXIV.

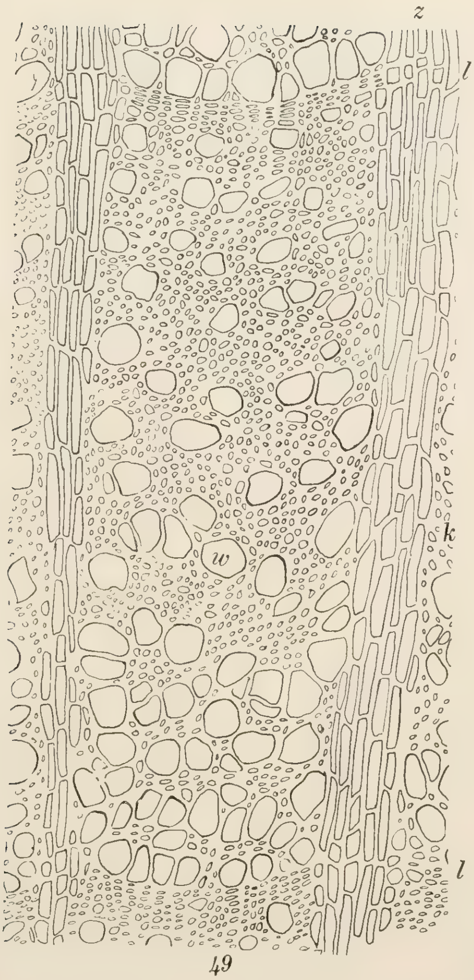
FIG. 49. Cross section of wood: *l*, *l*, limits of a year's growth; *z*, medullary ray; *w*, tracheal tube; *k*, fiber tracheids. × 85.

FIG. 50. Longitudinal section of wood: *z*, medullary rays; *w*, fiber tracheids. × 85.

FIG. 51. Cross section of wood: *z*, medullary rays; *w*, tracheal tube; *k*, fiber tracheids; *v*, wood parenchyma. × 155.

FIG. 52. Longitudinal section of wood. Lettering same as in fig. 51. × 155.

PLATE LXXIV.



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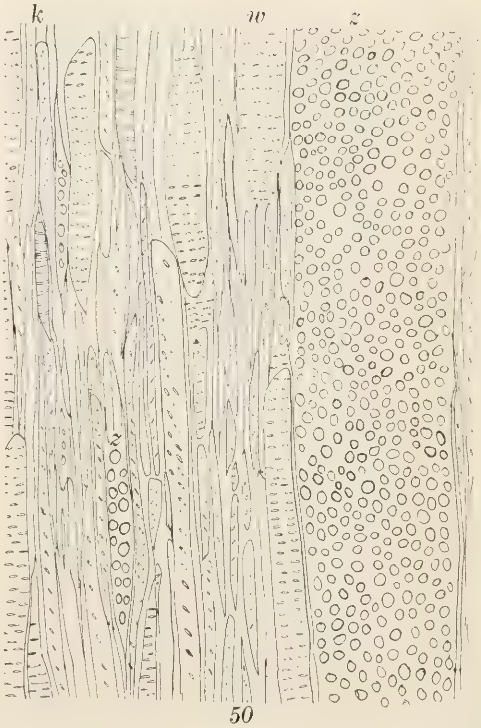

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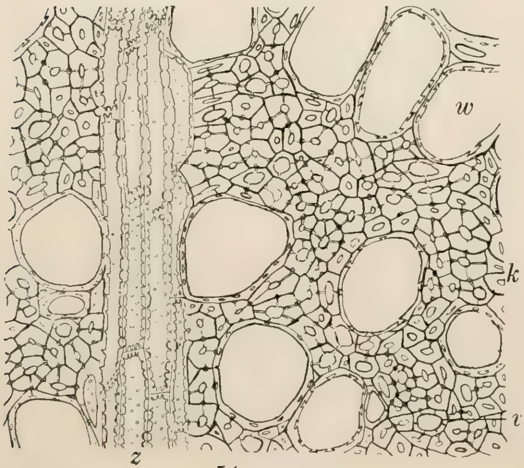

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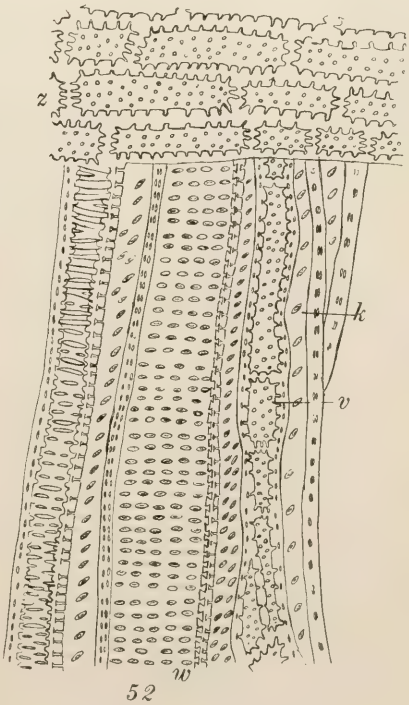

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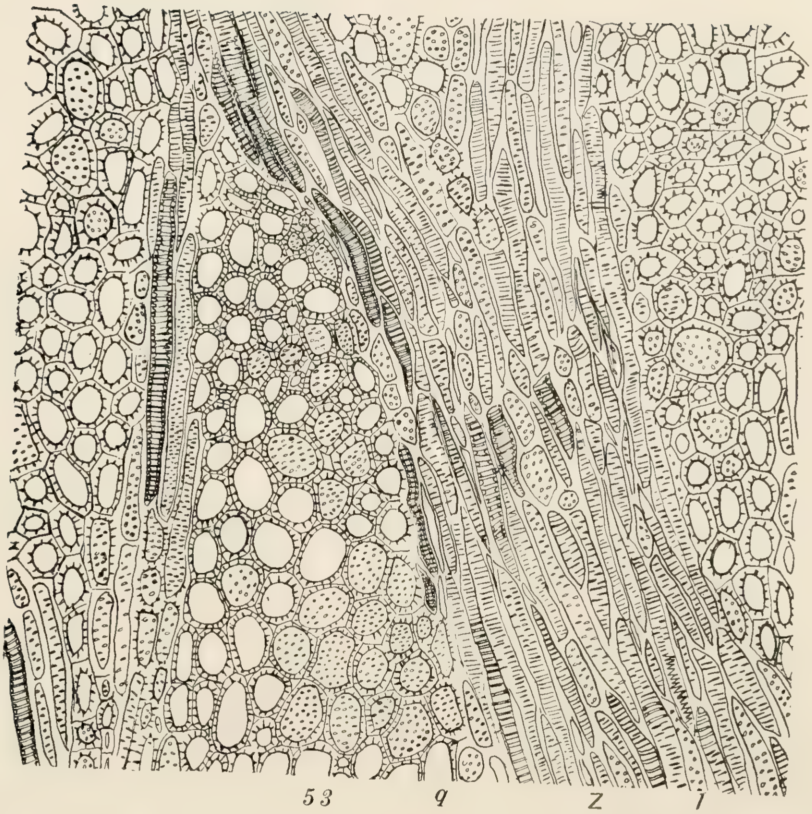
PLATE LXXV.

Fig. 53. Longitudinal section of the wood under the terminal bud: *q*, stone cells; *z*, tracheids; *l*, wood parenchyma. × 260.

Fig. 54. Tangential section of the wood showing end view of medullary rays and their frequency per square millimeter. The square represents a square millimeter: *m*, medullary ray. × 21.

Fig. 55. A bast fiber from the wood. × 137.5.

PLATE LXXV.



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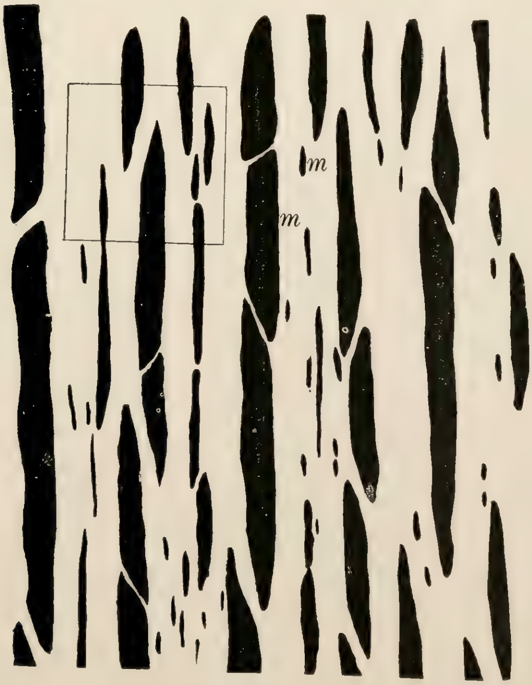

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PLATE LXXVI.

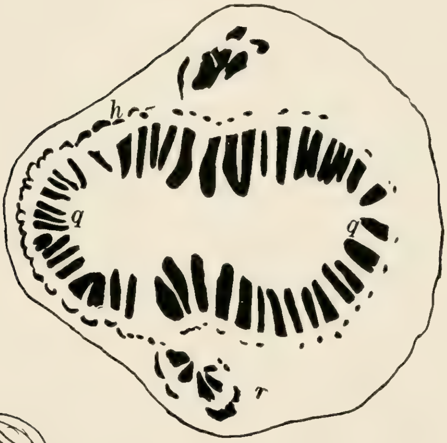
FIG. 56. Cross section of stem below terminal bud: *q*, wedges of xylem of the vascular bundle system; *r*, leaf trace; *h*, bundles of bast fibers; *l*, medullary rays. × 18.

FIG. 57. Longitudinal section of the stem below the terminal bud, showing the anastomosing of the vascular bundles: *l*, medullary ray; *q*, vascular bundle; *h*, bundle of bast fibers. × 18.

FIG. 58. Longitudinal section through old and new growth of stem: *j*, xylem of new growth; *l*, xylem of the last year's growth; *h*, bundle of bast. × 18.

FIG. 59. Longitudinal section of pith: *d*, pith of the last year's growth; *c*, pith of current year. Stippling indicates cell contents. × 173.

PLATE LXXVI.



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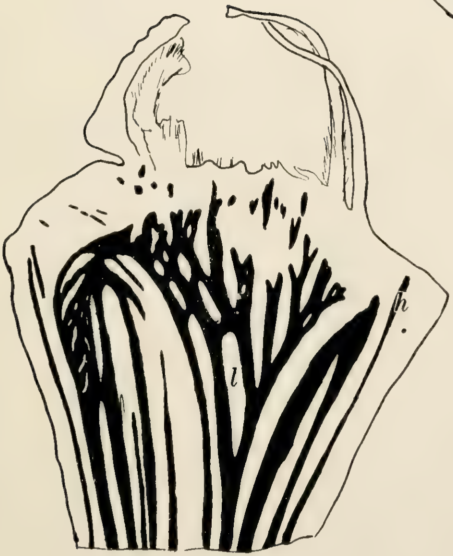

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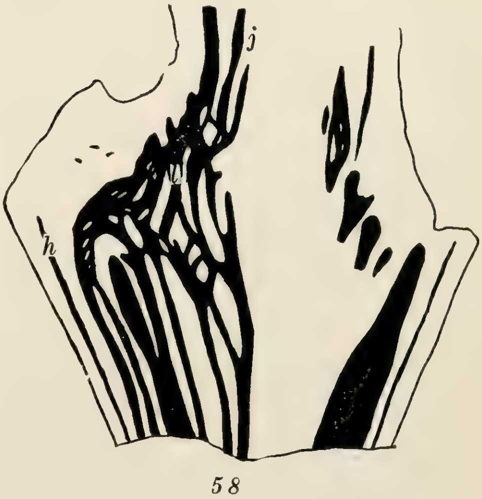

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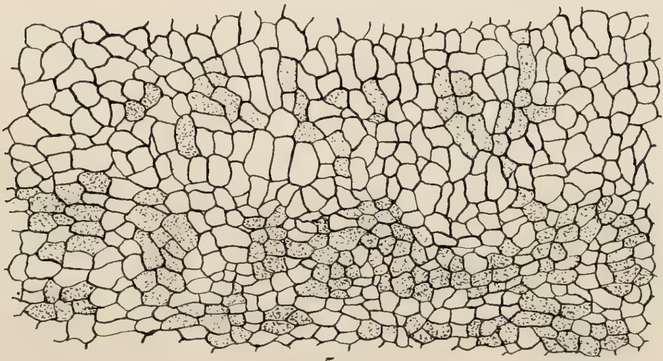

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